Genetic epidemiology of hereditary and congenital anomalies in the population of Mandi Bahauddin

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In the name of Allah, the most beneficent and merciful.

Declaration

I hereby declare that the work accomplished in this thesis is the result of my own research work carried out in Human Genetics Lab, Department of Zoology, Quaid-i-Azam University Islamabad. The epidemiological study was carried out in district Mandi Bhauddin. This thesis has neither been published previously nor does it contain any material from the published resources that can be considered as a violation of international copyright law. Any part or content of this thesis if copied from any source has been properly mentioned with reference to the source of citation.

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Urwa Hafeez

Dedication

This dissertation is dedicated to my

Father, Hafeez Ahmed

For supporting me unconditionally, believing in me,and without whom, I would never have completed this work

And

Mother, Kalsoom Akhtar

For her all prayers and love

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Abbreviations

CA	Congenital anomalies
CMV	Cytomegalovirus
CNS	Central nervous system
CP	Cerebral palsy
CVS	Chorionic villus sampling
EUROCAT	European Surveillance of Congenital Anomalies
HIV	Human Immunodefeciency Virus
HSV	Herpes Simplex Virus
ICD	International classification of disease
MRI	Magnetic resonance imaging
NMDs	Neuromuscular diseases
NTD	Neural tube defects
OMIM	Online Mendelian Inheritance in Man
TORCH	Toxoplasmosis,Others, Rubella, Cytomegalovirus, Herpes simplex
VZV	Varicella-Zoster Virus
WHO	World Health Organization
ZIKV	Zika Virus

Abstract

Congenital anomalies (CA) also known as birth defects are functional, structural, and metabolic defects that occur during the period of organogenesis and observed at birth or later in life. CA are caused by a mutation in a gene, chromosomal aberrations, environmental factors, micronutrient deficiencies and multifactorial effects. CA causes significant mortality and morbidity among children both in developing and developed countries. Data on the prevalence-pattern of CA from the rural populations of Pakistan is largely missing. In this context, the main objective of this study was to investigate the genetic epidemiology and phenotypic pattern of CA in the population of Mandi Bahauddin. During this cross-sectional epidemiological study, a total of 201 independent cases with CA were recruited from door-to-door surveys. All the anomalies were clinically diagnosed by physicians and specialized doctors and were classified into four major categories. In this cohort, the prevalence of the birth defects was in the following order: sensorineural defects (58%), neurological disorders (20%), neuromuscular anomalies (16%), and visual impairments (6%). Intellectual disability was the most common neurological disorder while cerebral palsy was most common neuromusculat anomaly. The representation of affected males (64%) was high as compared to affected females (36%). The sporadic cases 60% (n=121) were abundant in comparison with familial cases 40% (n=80). Majority of subjects were rural dwellers (54%) and belonged to lower middle class (42%). This study indicated a higher proportion of CA in young age group (56%). Parental consanguinity was found in 63% of the cases and familial cases had 40 % consanguineous marriages. Out of 80 familial cases,for 46%(n=37) subjects, affected persons were affected present in 2 generations and 64% (n=51) families had two family members affected.There was preponderance of subjects with first parity (40%).The current study provides useful information about the prevalence pattern of CA in Mandi Bhauddin and may bridge the gap in current knowledge about the causes and consequences of CA which may help relevant government authorities about the inception of management plans and health care centers for infants with CA.

1.1 Congenital anomalies

Congenital anomalies (CA) encompass a wide range of conditions that originate before or during birth. These anomalies can stem from genetic factors, including mutations in single genes and abnormalities in chromosomes. Additionally, environmental factors such as nutritional deficiencies, exposure to teratogens, and infections can contribute to CA Ssize, or location of any part, organ, cell, or its component in a child (Hudgins and Cassidy, 2006). These anomalies encompass both structural defects and molecular or cellular disruptions that are present at birth. Each year, generally 8 million infants are born with significant CA, with 3.3 million not surviving, and 3.2 million surviving but potentially experiencing disabilities later in life (Carmona et al., 2005). Any insult occurring between the 3rd and 8th week of gestation can result in a birth defect since this is a critical period for the normal development of organs (Malla, 2007). Birth defects typically arise during the period of organogenesis, which takes place between the 3rd and 8th week of pregnancy (Raza et al., 2012). These anomalies result from the interplay between genetic factors and environmental influences, and their prevalence varies across different geographical locations and over time (Ajao and Adeoye, 2019). If not adequately addressed, these conditions can lead to enduring physical, visual, cognitive, and auditory impairments (Abdou et al., 2019).

Congenital anomalies encompass two main types: developmental and structural. Structural anomalies primarily impact specific body parts, whereas developmental anomalies affect bodily functions, learning abilities, or sensory perception in individuals. Some cases involve both developmental and structural abnormalities, such as Down syndrome and spina bifida. Structural anomalies can be further divided into two groups: minor and major anomalies. Minor anomalies typically do not cause severe

health issues during the neonatal period and have fewer long-term implications. On the other hand, major anomalies significantly affect the health of newborns and the overall life of the affected individual, often requiring medical interventions and surgeries (Anele et al., 2022).

Congenital anomalies can either be familial or sporadic depending on the pattern of inheritance. The term "familial" describes genetic disorders that run-in families, occur more frequently in each family, and can be passed down to future generations, whereas the term "sporadic" describes disorders that do not run in families, occur in an irregular pattern, and cannot be passed down to future 5 generations (Hemonta et al., 2010). Genetic disorders can either be isolated or syndromic depending on which organs or body parts are affected. In isolated genetic disorders, only a single organ or parts of the body are affected while in syndromic various organs or the body's parts are affected (Sadler et al., 2019).

The consequences of congenital anomalies vary widely, with a significant spectrum of severity. The presence of congenitally abnormal infants not only causes emotional and psychological distress for parents but also raises concerns among medical specialists (Connolly et al., 2014). Long-term handicap brought on by congenital anomalies can have a profound impact on people, families, healthcare systems, and societies. Congenital anomalies continue to be stressful for the child-bearer and the entire family who have waited long enough to hold a normal kid. Further, caring for an atypical child, such as one with cleft-lip or palate, can be highly traumatic for the family (Arijo et al., 2022). However, comprehensive investigations into the epidemiology, risk factors, and distribution of CA are necessary before implementing intervention efforts. Managing congenital anomalies is challenging and necessitates a comprehensive understanding of the problem.

1.2 Worldwide prevalence of CA

The measurement of the overall prevalence of birth defects presents a challenge due to factors such as prenatal losses, including blighted ovum, ectopic pregnancies, and miscarriages. Previously, the term "birth prevalence" was used. However, advancements in prenatal diagnostic techniques have led to the prognosis of fetal abnormalities, resulting in the termination of pregnancies if the defect is deemed severe. As a result, the rate of birth defects is calculated by considering live-born infants, stillborn fetuses, and prenatally diagnosed or terminated abnormal fetuses (Czeizel, 2005). The global prevalence of congenital anomalies is estimated to be around 3-7%, but it can vary from country to country. Each year, 7.9 million children, accounting for 6% of all children born worldwide, are born with an anomaly (Carmona, 2005). The worldwide prevalence of congenital anomalies varies significantly from country to country, as evidenced by various studies. In low and middle-income countries, the rates of birth defects tend to be higher due to environmental teratogenic risk factors compared to high-income countries (Mekonnen et al., 2020). These congenital anomalies are more frequently reported in low and middle-income countries, accounting for over 90% of cases (Ajao and Adeoye, 2019).

In the United States (US), the prevalence of CA ranges from 3% to 5%, while in Europe, it is 2.1% (EUROCAT, 2015). Studies conducted in India have found that 2.5% of newborns exhibit both minor and major defects, making birth defects the third most common cause of perinatal losses in the country (Marwah et al., 2014). The overall incidence of congenital anomalies in India is estimated to be around 2%-5%. In China, over 0.8-1.0 million CA cases occur annually, meaning a newborn with a birth defect is

observed every 30-40 seconds (Zheng et al., 2004). Other countries also experience varying prevalence rates of CAs. Japan has a birth prevalence of 1.07%, while Taiwan's rate is 4.3%. In England, the birth prevalence is 2%, and in South Africa, it is 1.49%. Lebanon reports a prevalence of 1.64% for major CA (Francine et al., 2014). In the UK, the prevalence of birth defects is 3.3%. Hospital-based studies in Nigeria show a prevalence range between 0.4% and 11.1% (Ajao and Adeoye, 2019). Sudan has a birth prevalence of CA at 82 per 1,000 live births, while France's prevalence is 39.7 per 1,000 live births. In Uganda, Nigeria, Kenya, Saudi Arabia, and Pakistan, the birth prevalence ranges between 20 and 30 per 1,000 live births (Abdou et al., 2019). In Nepal, congenital anomalies were reported in 52 per 10,000 children and were responsible for 7% of all neonatal deaths (Khanal et al., 2019). In Korea, among babies with birth defects, the infant mortality rate was 6.8 per 10,000 live births (Kurdi et al., 2019). Tanzania has an estimated prevalence of 60.5 per 1,000 live births (Kishimba et al., 2015). The type and frequency of birth defects can vary significantly in different populations due to factors such as socio-economic status, ethnicity, environmental influences, nutrition, lifestyle, and maternal age, among other reasons (Kumar et al., 2021).

As per WHO Fact Sheet, of a total of 2.68 million neonatal deaths in 2015, 303,000 were attributed to congenital anomalies. Preterm birth complications accounted for 9,47,000 deaths, while birth asphyxia or birth trauma caused 6,37,000 deaths. Neonatal sepsis and other factors contributed to 4,01,000 and 3,92,000 deaths, respectively (WHO, 2016).

1.3 Status of CA in Pakistan

Pakistan is characterized by its diversity, with a dozen linguistic families, including Sindhi, Pashto, Balochi, Saraiki, Hindko, Urdu, Potohari, Kashmiri, Brahui, and Shinai, forming the ethnic majority of the country. Consanguineous marriages are widespread in the region due to various beliefs, such as the importance of preserving ancestral land within the family, strong family bonds, better compatibility, lower divorce rates, and simplified marriage contracts (Jabeen and Malik, 2014). Moreover, certain religious groups, like the Shias, abstain from marrying outside the family due to their strict religious principles (Bennett et al., 2002). In more remote areas of Pakistan, the caste and tribal systems play a significant role, leading to numerous inter-family marriages, particularly prevalent among the Arain people residing in Punjab province. Regarding the status of congenital anomalies (CA) in Pakistan, there is a lack of specific country-wide estimations. However, certain community-based studies have been conducted to assess the prevalence of perinatal mortality (Maqbool and Bhutta, 2002). Although prevalence studies have been conducted in private sector institutes, there is a lack of data on sequential time trends and risk factors for perinatal mortality from public sector institutes. The occurrence of congenital anomalies (CA) in Pakistan is notably high, attributed to factors such as large sib ships, low socio-economic status, the common practice of consanguineous marriages, and maternal influences. These CA contribute to 6-9% of prenatal deaths (Bibi et al., 2022) and account for 2% of the total death toll in Pakistan (Bhatti et al., 2019). Studies conducted in Pakistan have revealed varying birth prevalence rates of congenital anomalies, ranging from as low as 1.4% to as high as 7% (Anbreen et al., 2021).

In Pakistan, the available data on perinatal mortality primarily comes from hospital-based studies. A multicenter clinical survey conducted by the Society of Obstetricians and Gynecologists of Pakistan (SOGP) reported the Post Neonatal Mortality Rate (PNMR) as 92 per 1000 live births, with stillbirths accounting for 72% of total births. In Pakistan, it is estimated that 6% to 9% of perinatal deaths are attributed to birth defects (Tirumani and Khatija,2017). According to the World Health Organization (WHO) report published in 2017, deaths related to birth defects reached 26,492, accounting for 2.17% of total deaths in Pakistan, with an age-adjusted death rate of 10.4 per 1,000, ranking Pakistan at number 59 globally.

1.4 Types of congenital anomalies

There are various types of congenital anomalies found in human populations, including neuromuscular anomalies, ear defects, neurological disorders, limb deformities, and visual impairments.

1.4.1 Hearing impairments

Hearing impairments, specifically congenital hearing loss, occur when the ability of the ear to convert sound vibrations into nerve impulses is disrupted at birth. This condition can be categorized based on the specific area of the ear that is affected. Conductive hearing loss refers to impairment in the outer or middle ear, while sensorineural hearing loss involves issues with the inner ear, auditory nerve, or central auditory pathway. Mixed hearing loss is diagnosed when both conductive and

sensorineural components are present (Boudewyns et al., 2011). Conductive hearing loss occurs when sound waves are unable to properly transmit through the ear due to malformation or underdevelopment of the middle or external ear, temporary blockages caused by fluid buildup, or other factors. Deafness, which is a severe form of hearing impairment, can have various causes including both environmental and hereditary factors. It manifests in diverse clinical and genetic forms.

In many developed countries, neonatal hearing-screening programs are of paramount importance as they facilitate the early detection of hearing impairments, enabling timely intervention to prevent delays in speech and language development. Early intervention also yields long-term positive effects on social and emotional development, ultimately contributing to an improved overall quality of life for affected individuals. When investigating cases of hearing loss, a thorough search for the underlying cause is typically conducted, leading to a definitive diagnosis. . In developed countries, genetic abnormalities are often the primary cause of hearing loss cases. Congenital hearing loss can be attributed to a combination of environmental and prenatal factors, which may be more prevalent in low-income areas. Moreover, certain congenital infections, with cytomegalovirus being a prominent example, are known to be significant contributors to hearing loss (Grosse et al., 2008).

Sensorineural hearing loss (SNHL) is a type of hearing loss that arises from issues with the central auditory structures or the vestibulocochlear nerve. Congenital SNHL can be attributed to genetic factors (either isolated or syndromic) or can result from sporadic insults during fetal development. When imaging is conducted, the majority of patients with congenital SNHL exhibit normal inner ear morphology and internal auditory canal structural features, although anomalies are detected in 24% to 41% of cases (O'Brien et al., 2021). Hearing loss is also classified based on the presence

or absence of a co-inherited physical condition. When a physical condition accompanies hearing loss, it is referred to as syndromic hearing loss, whereas hearing loss without any associated physical condition is known as non-syndromic hearing loss (Smith et al., 2005).

1.4.2 Neurological disorders

Worldwide studies indicate that central nervous system disorders are frequently encountered in CA. A notable neurological disorder is intellectual disability, which is discussed here.

Intellectual disability (ID) is a mental health condition characterized by impaired cognitive function, leading to significant developmental deficiencies in learning, problem-solving, adaptive skills, and independence. These deficiencies typically arise before the age of 18, persisting throughout the individual's life (Lévy et al., 2018). The severity of functional impairment in ID can vary and is measured using the intelligence quotient (IQ) score, which ranges from mild to severe. While genetic factors are increasingly recognized as major contributors to ID, the causes of the condition are highly diverse (Gilissen, 2014). Intellectual disability (ID) is influenced by a combination of factors, with genetic factors playing a significant role. Aneuploidies, copy number variations (CNVs), and mutations in specific genes can contribute to genetic variability and may lead to various ID-related conditions, such as X-linked ID (XLID), ataxias, motor neuron disease, and epilepsy (Zablotskaya et al., 2018). The diagnosis of ID involves identifying clinical phenotypic characteristics, such as delayed speech, hypotonia, and seizures, which can help healthcare

professionals determine the specific nature and severity of the condition (Redin et al., 2014).

Intellectual disability is a chronic condition that affects individuals throughout their lives. While there is currently no cure, many people with ID can improve their functionality over time through various treatment strategies. Treatment approaches typically focus on the person's strengths, addressing their specific needs, providing necessary support, and managing any co-existing conditions (MNT, 2021).

1.4.3 Neuromuscular anomalies

Neuromuscular anomalies refer to conditions that impact on the nerves responsible for controlling voluntary muscles and transmitting sensory information to the brain. Neurons, which are nerve cells, play a vital role in sending and receiving electrical messages to and from the body, enabling the control of voluntary muscles. However, when neurons become unhealthy or die, communication between the nervous system and muscles is disrupted. This disruption results in muscle weakness and waste, a condition known as atrophy. Among the various neuromuscular anomalies, one of the most common disorders is cerebral palsy (CP) (Cedars Sinai, 2021).

Cerebral palsy (CP) is a complex neurological disorder that can manifest in various forms, affecting both mental and physical functions, as well as specific aspects of gait, cognition, growth, and sensation. The condition is characterized by static brain damage that occurs before the completion of cerebral development. This brain damage leading to CP can occur during the prenatal, perinatal, or postnatal periods, as brain growth continues during the first two years of life (Krigger, 2006). It is considered a

multifactorial disorder, often resulting from brain injury before or during birth. While CP itself is non-progressive, the clinical expression of the disorder can change over time as the brain matures (Gulati and Sondhi, 2018). The majority of CP cases, 70%-80%, occur before birth, and the exact causes are largely unknown. Birth complications such as hypoxia are believed to contribute to around 6% of individuals with congenital CP. After birth, CP affects 10-20% of children primarily due to brain injuries caused by factors like bacterial meningitis, viral encephalitis, hyperbilirubinemia, and accidents (Thompson etal., 2001). Early intervention, supportive care, and appropriate therapies can greatly improve the quality of life for individuals living with CP.

1.4.4 Visual impairments

Visual impairment refers to a functional limitation in the eye or the visual system, resulting in a person's inability to see adequately. The classification of visual impairment by the World Health Organization (WHO) is based on two primary factors: visual acuity, which measures the clarity of vision, and visual fields, which assess the extent of the area from which an individual can perceive visual information when looking directly at an object. Additionally, photophobia, the sensitivity or intolerance to light, is often associated with visual impairment. According to the Center for Disease Control and Prevention (CDC) and the World Health Organization, low visual acuity is defined as eyesight ranging between 20/70 and 20/400 with the best available correction, or a visual field of 20 degrees or less. If the visual acuity is below 20/400 with the best available correction or the visual field is narrower than 10 degrees, it is considered blindness. In South Asia, Pakistan ranks third in terms of the prevalence of blindness and vision impairment, following India and Bangladesh. It is estimated that

there are a total of 21.78 million individuals of all ages affected by visual impairment in Pakistan (Mandal, 2021).

EUROCAT is a network of population-based congenital anomaly registries in Europe, which includes around 20 non-EU nations and covers 29% of the EU birth population. The primary purpose of EUROCAT is to record and monitor significant congenital anomalies occurring in the registered populations. However, minor anomalies and conditions that are inadequately described or connected to prematurity at birth may not be included in the registry (Boyle et al., 2018). Over nearly 30 years, the 25-population-based EUROCAT registries have identified a total of 250,000 congenital abnormalities among 11.5 million births across Europe. This comprehensive data collection and analysis helps in understanding the prevalence, trends, and impacts of congenital anomalies in the region (Loane et al., 2011).

1.5 Causes of CA

Recognizing the underlying causes of congenital anomalies (CA) is crucial for prevention and genetic counseling, but it proves challenging due to the multifactorial nature of these conditions (Hatch et al., 2018). CA cases with unknown origin are 40 to 60%, with genetic factors accounting for 30 to 40%, and environmental influences contributing to 5% to 10% (Tayebi et al., 2010). Within the realm of genetic causes, chromosomal abnormalities account for 6% of cases, single-gene disorders for 25%, and multifactorial factors for 20% to 30%. The higher overall occurrence of congenital anomalies associated with genetic factors can primarily be attributed to two factors: (i) women giving birth after the age of 35 and (ii) a significant prevalence of

consanguineous marriages (Czeizel, 2005). When estimating the contribution of genetic disorders to structural malformations, it is essential to consider the timeframe in which these anomalies are detected, and the genomic technology employed. The genetic patterns of congenital anomalies in newborns who survive may differ from those observed in pregnancies that result in spontaneous abortion or are not continued beyond the 22nd week, as several morphological anomalies lead to such outcomes (Heinke et al., 2020). In contrast to the average 36-50% of cases with structural anomalies explained in studies focusing on stillbirths using genome sequencing, the population examined by Holmes and Nelson encompassed both live-born and miscarried infants. Their estimation indicated that around 12% of cases with structural anomalies could be attributed to a specific responsible gene (Quinlan-Jones et al., 2019).

1.5.1 Single gene disorders

Single-gene disorders are easily understandable due to their straightforward inheritance patterns, such as recessive or dominant traits (Boyle, 2017). These disorders result from mutations in either one or both alleles of an autosome, X-chromosomes, or the mitochondrial gene (Fink et al., 2018). They are also known as monogenic disorders, and thousands of them have been identified, with various inheritance methods, including autosomal dominant, autosomal recessive, and X-linked patterns (Blencowe et al., 2018).

In autosomal dominant inheritance, only one copy of the faulty gene (from either parent) is required to cause the disorder. On the other hand, autosomal recessive disorders necessitate both parents passing on a faulty copy of the gene to the affected offspring, as seen in conditions like sickle cell anemia, cystic fibrosis, and Marfan

syndrome (Davis, 2019). Single-gene disorders present significant health challenges and are widespread in various regions worldwide. Some common examples include sickle cell anemia, cystic fibrosis, Tay-Sachs disease, hemophilia, and color blindness, among others.

1.5.2 Multifactorial disorders

Multifactorial disorders result from the intricate interplay between both hereditary and environmental factors. The extent to which hereditary and environmental elements contribute varies among different disorders. The development of multifactorial disorders hinges on predisposition, which elevates an individual's risk level and makes them susceptible to the influence of environmental factors (Rodriguez-Vieitez ,2016). Some examples of multifactorial disorders include cancer, depression, cardiovascular diseases, and endocrine disorders (Stolk et al., 2007).

1.5.3 Chromosomal abnormalities

Chromosomal abnormalities arise from both structural and numerical changes in chromosomes. Numerical changes occur due to non-disjunction during meiosis, resulting in aneuploidy, where an individual receives an extra chromosome or a fewer number of chromosomes. This imbalance in chromosome numbers leads to congenital anomalies in the fetus (Natarajan, 2002). Structural changes in chromosomes occur due to deletions, insertions, and rearrangements of chromosomal segments. More severe effects can be observed with sex chromosome additions or deletions. For instance, Turner syndrome is caused by the absence of an X chromosome in a female embryo

(XO), while Klinefelter's syndrome results from an extra X chromosome in addition to the typical combination of one X and one Y chromosome in a male baby (XXY) (Blackburn, 2017). A well-known example of chromosomal abnormality is Down's syndrome, which is caused by an extra chromosome and referred to as trisomy 21. Other examples include Edward's syndrome and Patau syndrome (Aliyu, 2021). Down syndrome can occur in all populations, but the number of live births affected may vary due to differences in the age of mothers at the time of conception (Antonarakis et al., 2021).

1.6 Risk Factors

Environmental factors play a significant role and encompass exposure to teratogenic agents, infectious agents, socioeconomic factors, and demographic factors, including maternal nutrition status (Verma, 2021). Low income indirectly contributes to the occurrence of CA, with a higher frequency observed among families and countries with limited resources. Most of severe CA cases (94%) are prevalent in low- and middle-income countries, where a higher risk is associated with a possible lack of access to sufficient and nutritious foods for pregnant women, as well as inadequate access to healthcare and screening (WHO, 2016). Risk factors contributing to birth defects can be categorized into two groups: those that can be modified or influenced (modifiable) and those that cannot be altered (non-modifiable). Modifiable risk factors can potentially be changed through patients' education and genetic counseling, providing possible avenues for prevention. In contrast, non-modifiable risk factors involve genetic elements such as age, ethnic background, consanguinity, maternal and

paternal age, which remain unchangeable or only marginally influenced (Harris et al.,2017).

The increased prevalence of congenital disorders is associated with various risk factors, including large family sizes, exposure to radiation, chemical substances, infectious agents, prematurity, medication usage, maternal illnesses, and occupational exposures. Environmental exposure may exert a mutagenic action before conception or have a teratogenic impact after conception (Taboo, 2012). Furthermore, in low and middle-income countries, maternal infections with syphilis and rubella are also reported as potential risk factors for congenital anomalies (Mekonnen et al., 2020).

Globally conducted prevalence studies consistently reveal a high occurrence of congenital anomalies in the offspring of consanguineous couples. Consanguineous marriages have been associated with increased incidences of abortion and stillbirth (Riaz et 5al., 2016). Additionally, the historical presence of inheritable congenital diseases, previous miscarriages, and stillbirths are significant factors in the etiology of birth defects (Ajao and Adeoye, 2019).

Moreover, certain risk factors associated with congenital anomalies include maternal use of recreational drugs, smoking, deficiencies in multivitamins and minerals, infectious viral diseases, maternal conditions like insulin-dependent diabetes mellitus, and the use of specific maternal medications such as thalidomide and anticonvulsants (Sarmah et al., 2016).

1.6.1 Consanguineous marriages

Consanguineous marriages refer to unions between close blood relatives, often involving first and second cousins in the context of medical genetics (Hamamy, 2012).

Such unions may lead to the transmission of two recessive faulty processes to the offspring, one inherited from the maternal side and the other from the paternal side, potentially resulting in the manifestation of congenital malformations (Kamal, 2020). Consanguineous unions are associated with a significantly higher incidence of abortion and stillbirth. Additionally, consanguineous communities exhibit elevated rates of reproductive losses, including post-neonatal, neonatal, infant, and pre-reproductive mortalities, when compared to non-consanguineous marriages (Riaz et al., 2016).

1.6.2 Folic acid deficiency

Insufficient intake of folic acid during pregnancy can lead to the occurrence of congenital anomalies such as anencephaly and spina bifida (Varunashree et al., 2017). Folic acid plays a crucial role in organ formation, particularly during the first trimester of fetal development, including the development of the neural tube. To support healthy fetal development, women of childbearing age require 0.4 to 0.8 mg of folic acid. In the absence of a diet rich in folate, mutations in the methylene-tetra-hydro folate reductase gene have been associated with elevated levels of maternal plasma homocysteine and an increased risk of neural tube abnormalities in children (Harris et al., 2017).

1.6.3 Air Pollution

Air pollution is becoming a significant risk factor for congenital anomalies. A higher level of exposure to PM10 (particulate matter,diameter 10 microns or less), resulting from vehicular traffic during pregnancy, has been linked to an increased

likelihood of birth defects. Among anomaly categories, musculoskeletal and chromosomal abnormalities show the most notable dose-response relationship with PM10 exposure (Taruscio et al., 2017). Numerous epidemiological studies and meta-analyses have indicated that exposure to ambient air pollution during pregnancy may potentially elevate the risk of congenital heart defects in newborns (Vrijheid et al., 2011). Consequently, greater attention should be devoted to monitoring ambient air pollution exposure in pregnant women (Sun et al., 2023).

1.6.4 Indoor environment

Most of the general population spends a significant portion of their lives indoors, where indoor air pollution becomes a significant concern. Exposure to organic solvents used in paint products within home environments during the first trimester of pregnancy has been linked to congenital anomalies. Hjortebjerg et al. (2012) conducted a study using data from the Danish National Birth Cohort, where they interviewed over 20,000 women in their 30th week of gestation regarding paint usage during pregnancy. They found that 7% of women had been exposed to paint fumes during the first trimester, and after accounting for typical confounding factors and occupational solvent use, exposure to paint fumes was positively associated with congenital anomalies related to the renal system. Indoor dust can contain toxic heavy metals like lead, especially in non-European countries. For instance, in countries such as China, indoor dust, drinking water, and outdoor emissions from industrial and mining activities can constitute a significant portion of the environmental lead burden, which may lead to congenital anomalies (Taruscio et al., 2017).

1.6.5 Exposure to pesticides

Human exposure to pesticides can happen through various routes, both environmentally and occupationally. Environmental exposure can occur through the consumption of food and water containing pesticide residues, while occupational exposure can happen during or after indoor/outdoor pesticide application (van den Berg et al., 2012). Such exposure has been associated with genetic and epigenetic modifications, as well as chronic diseases (Mostafalou and Abdollahi, 2013). Epidemiological studies have shown connections between pre- and post-natal exposure to pesticides and a range of adverse health outcomes, including fetal death, neurological deficits, childhood cancers, intrauterine growth restriction, preterm birth, and birth defects (Weselak et al., 2007). While the majority of congenital anomalies (CA) cannot be attributed to a specific cause, prenatal indoor exposure to pesticides and herbicides has been suggested to elevate the risk of teratogenicity due to the heightened susceptibility of fetal systems during specific periods of development (Stillerman et al., 2008). Furthermore, residence in areas with high pesticide use and conception during the spring season have emerged as additional risk factors for CA. Pesticide residues tend to be highest in surface and ground water during the growing seasons, which are typically the spring and early summer months. Consequently, spring conception has been linked multiple times to an increased risk of CA, particularly in rural areas where pesticides are extensively used during that season (Waller et al., 2010).

1.6.6 Advanced maternal age

Advanced maternal age poses an increased risk for certain genetic conditions in the fetus (Katherine, 2016). Notably, for human autosomal trisomies such as trisomy 21, advanced maternal age during conception is a significant contributing factor. This heightened risk can be attributed to the non-disjunction of homologous chromosomes or chromatids that occurs during the meiotic divisions responsible for oocyte formation (Antonarakis et al., 2021). The majority of chromosomal abnormalities arise from non-disjunction during meiosis 1. Maternal meiosis 1 initiates while the fetus is still in the ovary and remains arrested until ovulation takes place, which may be many years later. This prolonged meiotic arrest increases the chances of aneuploidy, as egg cells are vulnerable to mutations during this extended period (Hassold, 2007).

1.6.7 Intrauterine infections

Infections that can lead to congenital malformations are collectively referred to as TORCH infections, which stands for Toxoplasma, Others, Rubella, Cytomegalovirus, and Herpes. The category labeled as "others" has expanded to include several viruses and bacteria known to cause neonatal diseases. During pregnancy, certain infections pose significant concerns, specifically those caused by the rubella virus, syphilis, cytomegalovirus (CMV), and herpes simplex virus (HSV). Additionally, there are other potential infectious diseases now recognized for their ability to cause congenital infections and associated malformations. These include parvovirus B19 (B19V), varicella-zoster virus (VZV), West Nile virus, measles virus, enteroviruses,

adenovirus, and human immunodeficiency virus (HIV). In more recent times, Zika virus (ZIKV) has also been identified as responsible for congenital disorders. Experiencing such infections during pregnancy, particularly during the first nine weeks, can lead to serious congenital abnormalities. For example, maternal infections like cytomegalovirus, VZV, or rubella can have significant impacts on the developing fetus (Baud et al., 2016).

1.6.8 Maternal health and health services

Risk factors related to maternal health that are associated with congenital anomalies often increase the likelihood of other adverse birth outcomes, including preterm birth, low birth weight, and neurodevelopmental issues. This, in turn, places a greater burden on health services (Whitworth and Dowswell, 2007). As most congenital anomalies are multifactorial in nature, there exists an intricate interaction between various risk factors and genetics. Maternal health tends to be poorer among families with limited resources and in countries where mothers may face multiple concurrent risk factors. These factors include imbalanced nutrition, poor living environments, unhealthy lifestyles, and exposure to infections (Taruscio et al., 2017). Alongside maternal health factors, various environmental and external factors are strongly suspected or proven to have detrimental effects on fetal development, leading to abnormal development (Gilbert-Barness, 2010).

1.7 Diagnosis of CA

Detecting congenital anomalies (CA) during prenatal stages is crucial to understand the prognosis and potential consequences of birth defects. While it may not be possible to prevent all birth defects, the incidence and severity of such anomalies can be reduced through appropriate preventive measures. The initial step in preventing birth abnormalities is promoting health education and awareness. In the United States, organizations like the March of Dimes, along with both public and private healthcare systems, work towards enhancing maternal and child health by providing educational resources and implementing multiyear strategic planning (Sarmah et al., 2016). Prenatal diagnostic techniques can be both non-invasive and invasive. Non-invasive approaches include ultrasound screening, magnetic resonance imaging (MRI), and nuchal translucency screening. On the other hand, invasive methods such as chorionic villus sampling (CVS) and amniocentesis are also used for prenatal diagnosis (Impey, 2012). However, it's important to note that these invasive procedures come with associated risk factors, including infection, miscarriage, or amniotic fluid leakages. These various approaches enable healthcare professionals to detect and manage congenital anomalies effectively (Wagner et al., 2019). In developed countries, 30% of prenatal deaths in children are attributed to congenital malformations, resulting in considerable infant morbidity. Therefore, prenatal diagnosis of CA plays a vital role in enabling informed decisions during pregnancy and planning for appropriate perinatal treatment (Todros et al., 2001).

1.7.1 Ultrasonography

Ultrasonography stands out as one of the most effective techniques for detecting congenital anomalies during prenatal care. It has the capability to identify 35 - 50% of major fetal anomalies with a high level of specificity ranging from 90% to 100%. Typically, ultrasonography is performed around the 19th to 21st week of pregnancy, as recommended The safety and noninvasive nature of ultrasonography make it a preferred choice for prenatal screening. Moreover, its real-time display feature, along with accuracy and reproducibility, adds to its value in detecting anomalies without causing any harm to the patient throughout the course of pregnancy (Whitworth et al., 2015).

1.7.2 Amniocentesis

Amniocentesis is a commonly used method for diagnosing chromosomal abnormalities during pregnancy. This procedure involves extracting amniotic fluid from the mother's abdominal wall and is typically performed between the 15th and 20th week of gestation. It's essential to avoid performing it earlier, as it may lead to fetal complications. However, one of the significant concerns with amniocentesis is the occurrence of early miscarriages, which is a serious problem. Spontaneous abortions associated with amniocentesis are 1% (Tara et al., 2016). To minimize risks, amniocentesis is usually conducted between the 14th and 16th weeks of gestation when the fetus is not very large, but there is an ample amount of amniotic fluid (Collins et al., 2012). Despite precautions, there is still about a 1% chance of pregnancy loss linked to amniocentesis, and this risk increases with early performance of the procedure (Gil et al., 2015).

1.7.3 Chorionic villus sampling (CVS)

Chorionic villus sampling (CVS) is a diagnostic procedure employed to detect fetal anomalies. It is particularly reliable in diagnosing chromosomal, biochemical, and molecular disorders, making it comparable to the diagnostic capabilities of second-trimester amniocentesis (Jenkins, 1999). Typically, CVS is performed between the 10th and 13th weeks of pregnancy. During the procedure, a needle is carefully inserted into the uterus to extract placental tissue, enabling the diagnosis of various fetal anomalies (Jones and Montero, 2021).

1.7.4 Nuchal translucency (NT)

Nuchal translucency (NT) screening is a highly sensitive and effective diagnostic test for detecting fetal chromosomal abnormalities. First-trimester NT ultrasound serves as an important tool in diagnosing various congenital anomalies, particularly for the detection of trisomy 21 (Down syndrome) (Guraya and Shaista, 2013). An increased thickness of the nuchal translucency is associated with a higher risk of abortion, fetal anomalies, and fetal deaths. Various congenital anomalies, including neurodevelopmental delays, have been linked to an increase in NT thickness (Roozbeh et al., 2017). The accuracy of NT screening outcomes relies on the skills of the examiner. In some cases, a thickened nuchal translucency may be observed even with a normal karyotype, indicating the possibility of chromosomal aberrations . The sensitivity of NT screening is generally higher during the 11th to 12th week of pregnancy (Ceausu et al., 2018).

1.8 Preventive measures against congenital and hereditary anomalies

Preventing congenital and hereditary anomalies is a priority, especially in developing countries. The World Health Organization (WHO) recommends various measures to address this concern. These include promoting epidemiological knowledge of congenital and hereditary anomalies, focusing on maternal nutrition and family planning, providing genetic counseling about hereditary conditions and congenital anomalies, implementing preventive programs, and raising public awareness about these issues (Penchaszadeh, 2002). Infants born with congenital anomalies often require costly medical assistance, and complete recovery is often unachievable (Tayebi et al., 2010). To promote healthy pregnancies and reduce the risk of anomalies, it is crucial for expectant mothers to adopt a healthy lifestyle. This includes avoiding drugs, alcohol, and smoking, as well as maintaining a balanced diet. While these behaviors can be challenging to follow, they have a significant impact on the course of pregnancies. Additionally, there is substantial evidence supporting the benefits of prenatal folic acid supplementation in reducing the risk of congenital anomalies (Sarmah et al., 2016). Implementing these preventive measures can contribute to better maternal and child health outcomes and reduce the incidence of congenital and hereditary anomalies.

1.9 Aims and objectives

The objectives of the present study are:

- To identify the spectrum of different congenital and genetic anomalies in a general population of the Mandi Bhauddin district, Punjab.
- To evaluate biodemographic risk factors associated with congenital anomalies in the Mandi Bhauddin district.
- Parental consanguinity and other parental variables associated with the CA.

2.1 Study Population

Mandi Bahauddin, located in the northern part of Punjab, specifically in the north-central region of the province, is surrounded by various geographical features. To the north, it is bounded by the Jhelum River, which serves as a demarcation between Mandi Bahauddin and Jehlam districts. To the west lies the Sargodha district, while the river Chenab lies to the south, separating it from the Gujranwala and Hafizabad districts. The eastern border is shared with the Gujrat district (Punjab Portal, 2016). The district occupies a central area known as Chaj Doab, situated between the Jhelum and Chenab rivers, with coordinates ranging from 30° 8' to 32° 40' N and 73° 36' to 73° 37' E.

The roots of Mandi Bahauddin can be traced back to 1506 A.D. when a Gondal Jat Chief named Bahauddin established a settlement named Pindi Bahauddin after migrating from Pindi Shah Jahanian to this region. However, the name was eventually changed to "Mandi" due to the presence of a grain market in the area. Over time, the town saw development in the early 20th century near the ancient village of Chak No.51, attracting Sikh, Hindu, and Muslim businessmen and landowners who settled there (History of Pindi Bhauddin,2009). It is often referred to as the "City of Lions." Subsequently, with the implementation of the Punjab Local Government Ordinance 2001, it was granted the status of a Municipal Committee.

The Municipal Committee of Mandi Bahauddin Tehsil is subdivided into three tehsils: Mandi Bahauddin, Phalia, and Malakwal, which together encompass a total of eighty Union Councils. The entire district covers an area of 2,673 square kilometers (1,032 sq mi). The tehsil headquarters of Phalia and Malakwal are situated at distances of 22.5 and 28.5

kilometers (14.0 and 17.7 mi) from Mandi Bahauddin, respectively (Pakistan Bureau of Statistucs,2017).

Situated at an elevation of approximately 220 meters above sea level, Mandi Bahauddin experiences a moderate climate with hot summers and cold winters. In the peak of summer, temperatures can reach up to 48 °C (118 °F) during the day, while in winter, the minimum temperature can drop below 3 °C (37 °F).

According to the 2017 Census of Pakistan, Mandi Bahauddin holds the 41st position among the largest cities in terms of population. Over a span of 19 years, the city's population has grown by more than 99.62%, reaching a total of 198,609 individuals. During the census, the district's overall population was recorded as 1,594,039, with 775,788 males and 818,056 females. Among the total population, 1,267,681 resided in rural areas, while 326,358 lived in urban areas. The majority of the population, approximately 99.57%, adheres to Islam as their religion, with a small Christian minority primarily concentrated in urban areas. In terms of languages spoken, Punjabi is the primary language, spoken by 97% of the population, followed by Urdu (2.5%), Pashto (0.5%), and Saraiki (0.5%) (Census, 2017).As for tourist spots or historical buildings, apart from the Rasul Barrage located on the River Jehlum between Jhelum District and Mandi Bahauddin, and the Gurudwara Bhai Bannu in Mangat, District Mandi Bahauddin, there are no significant attractions in this district.

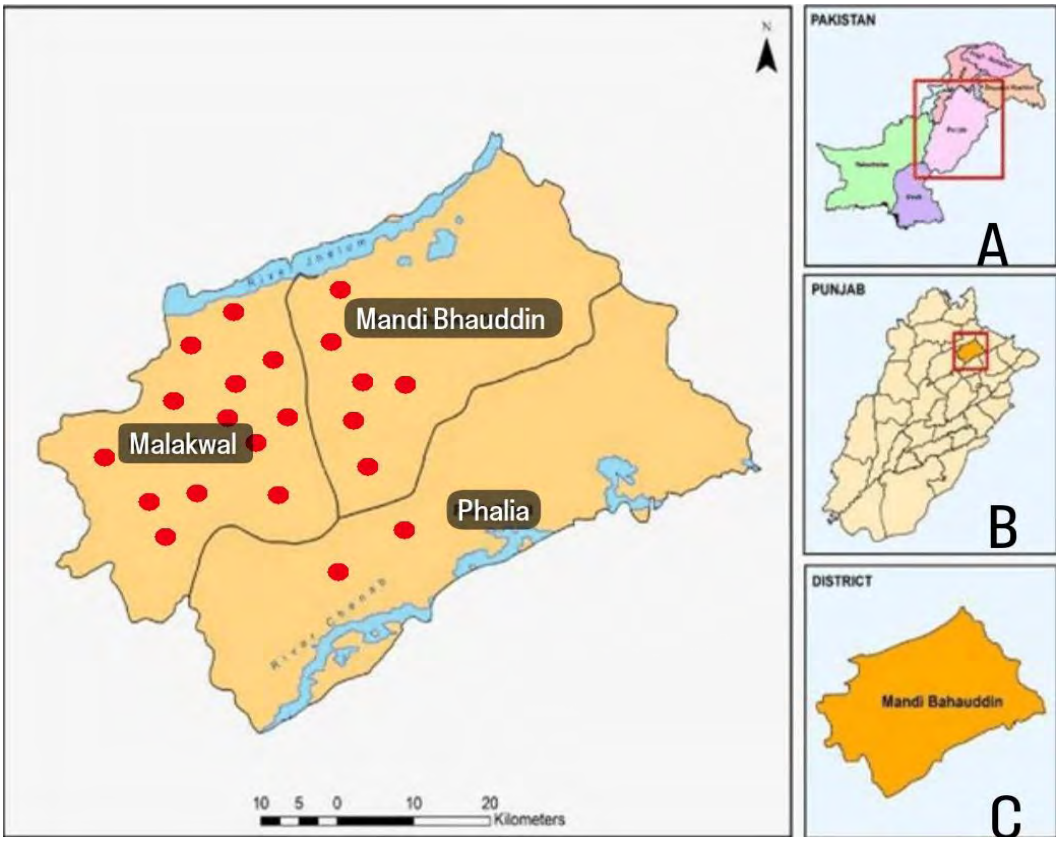


Fig. 2.1 (A). Map of Pakistan, (B). Punjab (C). District Mandi Bhauddin (relevant study area)

Source: https://www.researchgate.net/figure/Study-area-of-District-Mandi-Bahauddin_fig1_336147761

The current study focuses on investigating the occurrence of congenital anomalies in Tehsil Malakwal, my hometown. In this region, there is a lack of awareness among the residents about the consequences of congenital disorders, including their prevention, care, and treatment. Many people are unfamiliar with the concept of consanguinity and hereditary anomalies, and as a result, they often perceive these serious disorders as common illnesses. It specifically includes genetic abnormalities that can be easily identified based on their physical characteristics, without the need for molecular diagnosis. The main objective of this study is to gain a comprehensive understanding and assessment of the prevalence of

genetic anomalies in the general population. Additionally, the study aims to raise awareness about congenital and hereditary anomalies, particularly in rural areas where the majority of the population resides and may have limited access to formal education. By shedding light on these issues, the study seeks to contribute to the overall understanding of genetic anomalies and improve healthcare practices in the region. It is crucial to address this lack of awareness and knowledge among the population, and as a researcher, it becomes essential to educate and inform people about these severe and life-threatening disorders.

Table 1 Demographic variables of district Mandi Bhauddin

Variables	Estimates
Area	2673 km^2
Population 1998	1,160,552
Population 2017	1,594,039
Male	775,788
Female	818,056
Sex Ratio	94.83
Rural Population	1,267,688
Urban Population	326,358
Tehsils	3
Population Density	596.35
Household Size	6.27
Annual Growth Rate	1.68
Literacy Ratio	68.63

Source: Pakistan Bureau of Statistics (2017)

2.2 Proforma designing

For data collection, a standardized questionnaire was designed, consisting of three parts. The first part aimed to gather demographic information and included both categorical and continuous variables such as age, gender, place of origin, cultural background, socioeconomic status, occupation, religion, language, education level, family type, and more. The next part of the questionnaire focused on family-related data. It included details about the marriage status, whether it was consanguineous (between blood relatives) or non-consanguineous, the number of family members affected by the anomalies, the number of siblings who were normal and affected, the age of the parents, and other relevant information about the family structure. The third and final section of the questionnaire captured clinical and phenotypic information. This part involved gathering anthropometric measures, such as height (taken while standing or sitting), weight, and measurements of the head, neck, and chest circumference. Additionally, information about the specific body parts affected by the anomalies was recorded. This section covered a range of anomalies.

In addition to the questionnaire, each case was also subject to physical examination. Pictures were taken, and medical records were obtained whenever available to complement the data collection process. The combination of questionnaire responses, physical investigations, and medical records ensured a comprehensive approach to gathering relevant information for the study.

2.3 Sampling sites and family recruitments

The data collection process involved conducting a comprehensive survey, which included door-to-door visits in both rural and urban areas. The survey utilized various resources, including the assistance of family members and permanent residents in the region. Special education centers in the district played a crucial role in recruiting families, as we contacted them and later visited their homes to gather data. Most of the data was collected from rural areas, where families could be randomly recruited for the study. However, in urban areas, recruiting participants posed certain challenges. Nonetheless, efforts were made to ensure a diverse sample. Each recruited patient underwent a thorough diagnosis by a medical practitioner or physician to identify any congenital or hereditary anomalies. After identifying subjects with such anomalies, the purpose of the survey was explained to the families, and verbal consent was obtained from either them or the subject. To gather information, a structured questionnaire was administered to the families or subjects. In addition to this, comprehensive physical examinations were conducted on the patients, and their body measurements were carefully taken and recorded. Detailed phenotypic features of the subjects were documented to ensure accurate classification and facilitate further analysis. Photographs were taken of each patient for reference.

Most of the information gathered from these sources focused on conditions such as deafness and muteness, eye diseases, neuromuscular defects, and brain illnesses, including mental disabilities. However, the study did not include metabolic problems that required clinical tests for diagnosis, such as coronary heart disease, diabetes, and hereditary susceptibility to specific ailments.

2.4 Ethical approval

In human studies, numerous moral and ethical constraints exist. Hence, to ensure the ethical conduct of the present study, an official approval letter was obtained from the Ethical Review Committee of the Department of Zoology at Quaid-i-Azam University in Islamabad. Throughout the data collection process, explicit verbal consent is obtained from the family or subject involved, covering the necessary information and physical examination.

2.5 Pedigree construction

A pedigree serves as a visual depiction of family information pertaining to genetic anomalies and plays a crucial role in identifying genetic diseases. For every subject included in this study, a pedigree is constructed, gathering information from the older members of the family. The pedigree provides detailed information about the subject's family, aiding in the prediction of disease inheritance patterns.

During the construction of the pedigree, different symbols are employed. Squares represent males, while circles represent females. Nonconsanguineous marriages are depicted with single horizontal lines, whereas consanguineous marriages are shown with double horizontal lines. Affected subjects are shaded to differentiate them from unaffected family members. A slanted line over a symbol indicates a deceased individual. The number of generations in the pedigree is represented using Roman numerals on the left side, while the proband can be identified with an arrow below their symbol. The accuracy of all the information is repeatedly verified to ensure a clear and accurate representation of the family tree.

2.6 Duration and the preliminary phase of the study

The fieldwork took place between September 2022 and February 2023. Since the data collection relied on conducting door-to-door surveys, multiple visits were scheduled based on the availability of the subject or their parent/guardian. Before commencing the field work, the study's goals and objectives were carefully defined and established. This step ensured a clear direction and purpose for the research.

2.7 Data storage and analysis

To facilitate analysis, the data was classified into four primary divisions, each representing different types of anomalies. Subsequently, the subjects were further assessed based on their intellectual level, speech problems, and associated anomalies. To manage the collected data efficiently, an Excel sheet was used for storage, ensuring organized and easily accessible records. The data entry process involved categorizing the information into various sections, including age, gender, marital status, occupation, economic status, family type, residential area, number of affected males and females, mortalities, and more. Furthermore, the distribution of anomalies was examined based on different genetic factors, such as familial/sporadic attributes, isolate/syndromic nature of the disease, affected generations, parental marriage type, affected sibships, subject's parity, disease onset, and disease staging. The collected data was then transformed into tabular form and graphs to facilitate further analysis.

For statistical analysis, GraphPad Prism software was employed to analyze the data and draw meaningful conclusions from the study's findings. By employing appropriate statistical tools, the study aimed to gain deeper insights and draw valid conclusions regarding

the occurrence and distribution of anomalies in the population based on various factors. Additionally, all patient photos, along with the subjects' names, tracking IDs, and proforma, were stored digitally for future reference.

2.8 Databases search and literature survey

The classification of anomalies was performed by considering minor and major physical features, medical reports, and a comprehensive review of literature. Local medical practitioners and specialized doctors at the district headquarters hospital assisted in the initial categorization of the anomalies. To ensure accurate classification for the current study, standard genetic databases such as Online Mendelian Inheritance in Man (OMIM) and International Classification of Disease (ICD-10; Version 2019) were consulted.

A total of 201 subjects (128 males, 73 females) with a certain type of congenital anomalies were recruited during the study. A standardized questionnaire was utilized to collect the data through door-to-door surveys conducted in the Mandi Bhauddin district of Punjab. The observed anomalies were divided into 4 major categories: sensorineural defects (n=115), neurological disorders (n=41), neuromuscular anomalies (n=33), visual impairments (n=12).Most of the data was gathered from rural areas, with a higher prevalence of affected males compared to females.

3.1 Demographic variables with respect to gender

3.1.1 Distribution of subjects with respect to origin

All the index persons were categorized with respect to demographic variables. Most of the index persons were from rural areas and males (n=76). Out of 128(64%) male, 76 subjects were from the rural areas and only 52 subjects were from the urban areas. Among 73(36%) females, 33 females were from rural areas and 40 female subjects were from urban areas. The distribution of subjects with respect to origin was statistically non-significant (Table 2; Fig. 3.1.1).

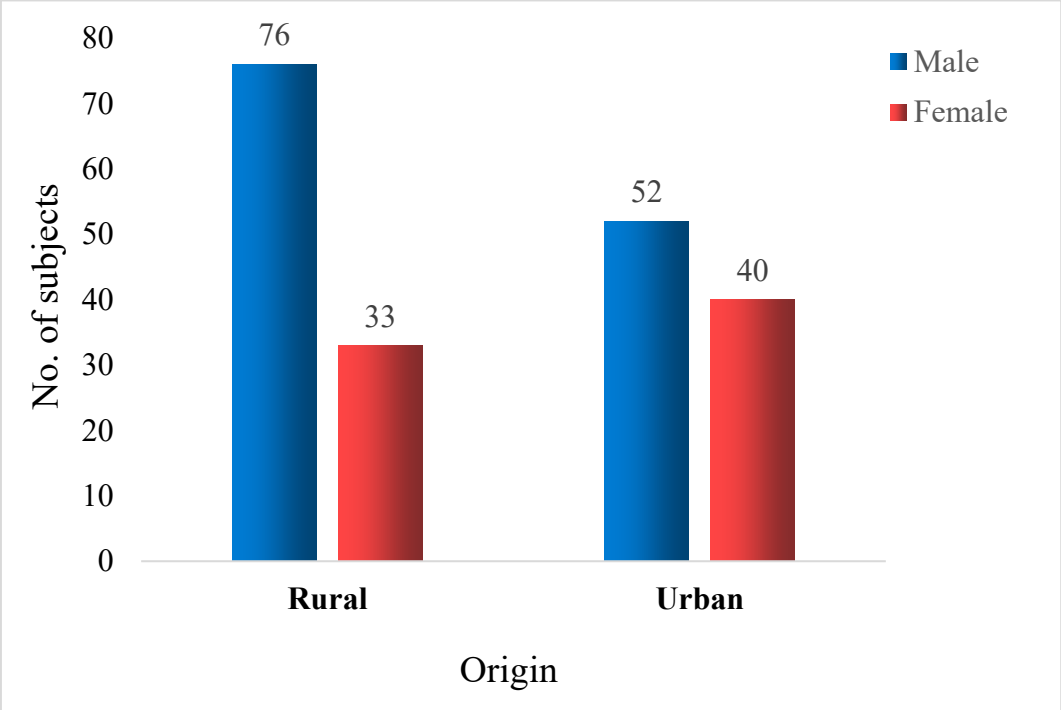


Fig.3.1.1 Distribution of subjects with respect to origin

3.1.2 Distribution of subjects with respect to age

Across all studied families, the age of affected subjects ranges between 3 years to 46 years. All the affected subjects were categorized into 4 major groups based on their age. In the first group, the subject's age ranges between less than or equal to 9 years and, a total of 31%(n=62) patients fall in this group. The second age groups range between 9-19 years and 56%(n=113) patients fall in this group out of which 73 subjects were male and females were 40 in number. This group has the highest number of patients. A total of 26 subjects fall in the remaining two groups. The distribution of subjects with respect to age was statistically non-significant (Table 2; Fig.3.1.2).

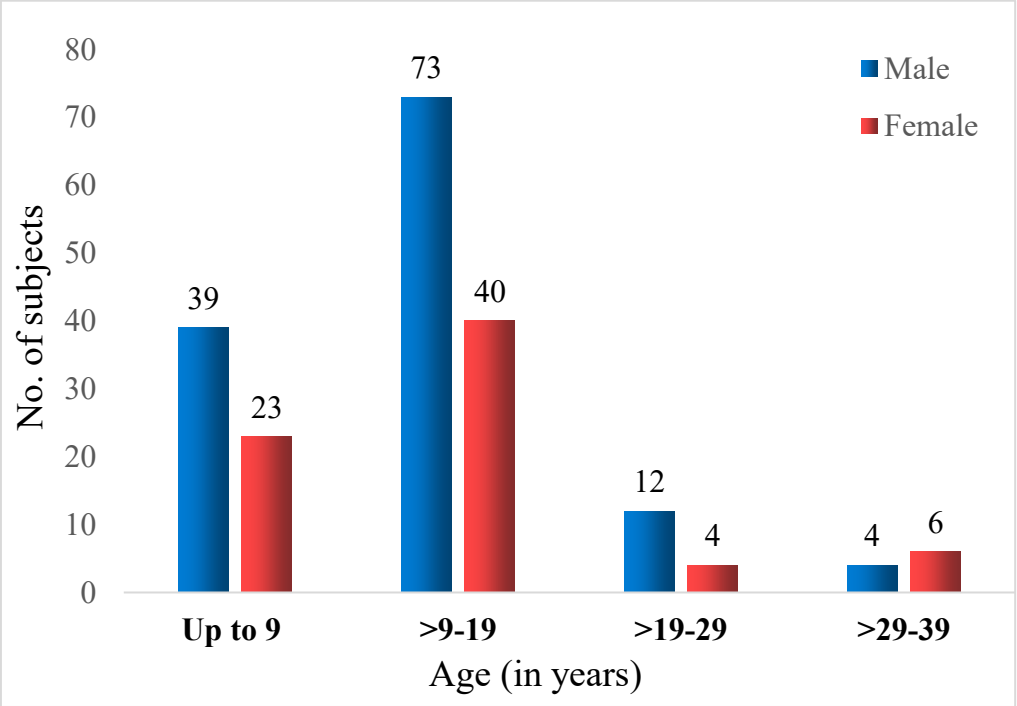


Fig.3.1.2 Distribution of subjects with respect to age

3.1.3 Distribution of subjects with respect to subject's education

Out of 201 total recruited families, 191 subjects are analyzed for literacy level of which 72%(n=137) subjects were literate and 29% (n=56) subjects were illiterate. Literate subjects were further classified into 2 groups that include primary and middle and, most of the subjects had primary schooling 83% (n=113). The distribution of subjects with respect to education was statistically nonsignificant (Table 2; Fig. 3.1.3).

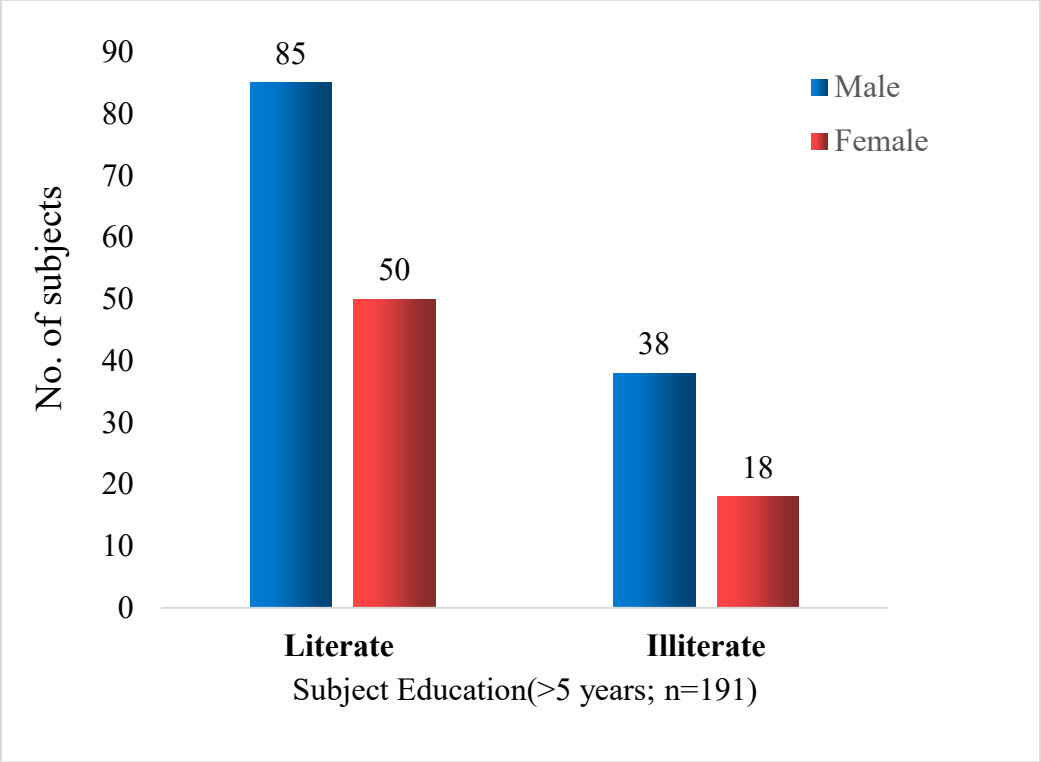


Fig.3.1.3 Distribution of subjects with respect to subject's education.

3.1.4 Distribution of subjects with respect to economic status

A total of 201 families were analyzed according to their economic status and further categorized into four classes as poor, low middle, middle and upper middle class. Most of the subjects belong to the lower middle class which was 42% (n=85) subjects. About 33% (n=67) of subjects belonged to the middle socioeconomic class and 21%(n=42) subjects were from poor class. Only 7 subjects belong to the upper middle socioeconomic class. The distribution of subjects with respect to economic status was statistically non-significant (Table 2; Fig. 3.1.4).

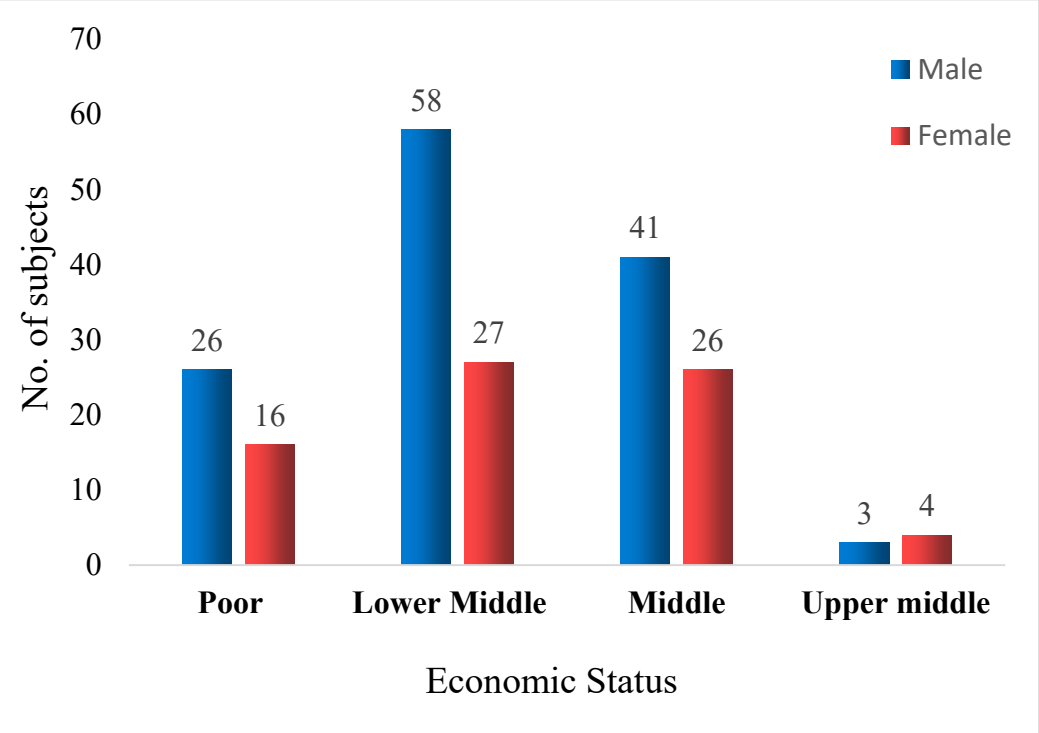


Fig.3.1.4 Distribution of subjects with respect to economic status

3.1.5 Distribution of subjects with respect to family type

All the subjects were categorized into two major family types one is nuclear and the other is extended. Most of the subjects 56% (n=112) belonged to the nuclear family system. The distribution of subjects with respect to family type was statistically non-significant (Table 2; Fig. 3.1.5).

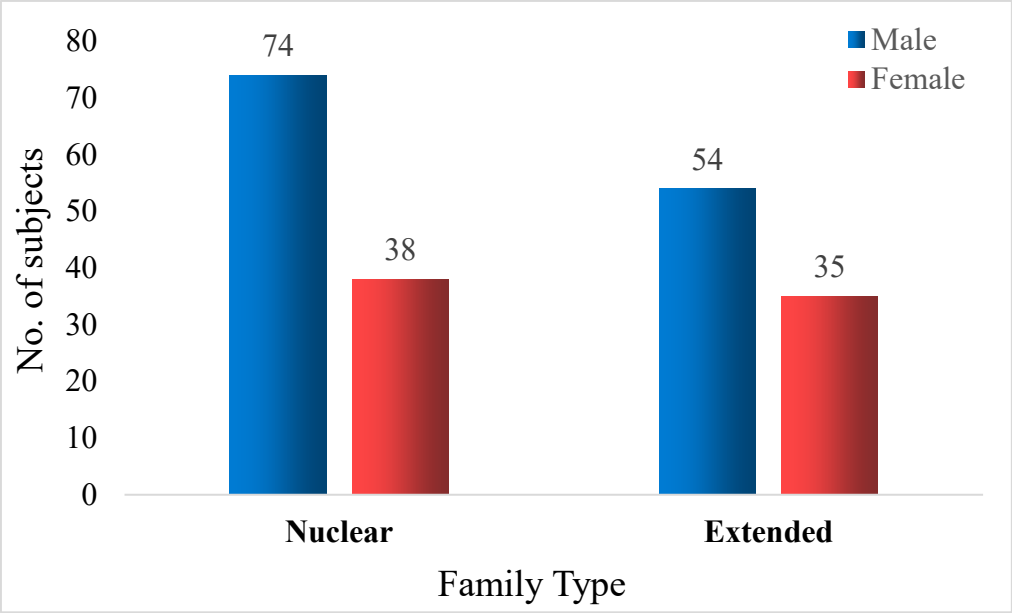


Fig.3.1.5 Distribution of subjects with respect to family type

3.1.6 Distribution of subjects with respect to caste

The subjects were further categorized with respect to their ethnicity, where the majority of subjects belonged to the Gondal ethnic group. A total of 18 subjects belong to the Syed caste. Among all, 15 subjects belong to the Khokar caste,13 subjects belong to Muslim Sheikh and 12 subjects belong to Awan caste. All other minor groups were marge

together into others and 113 patients fall in this group. The distribution of subjects with respect to caste was statistically significant (Table 2;Fig 3.1.6).

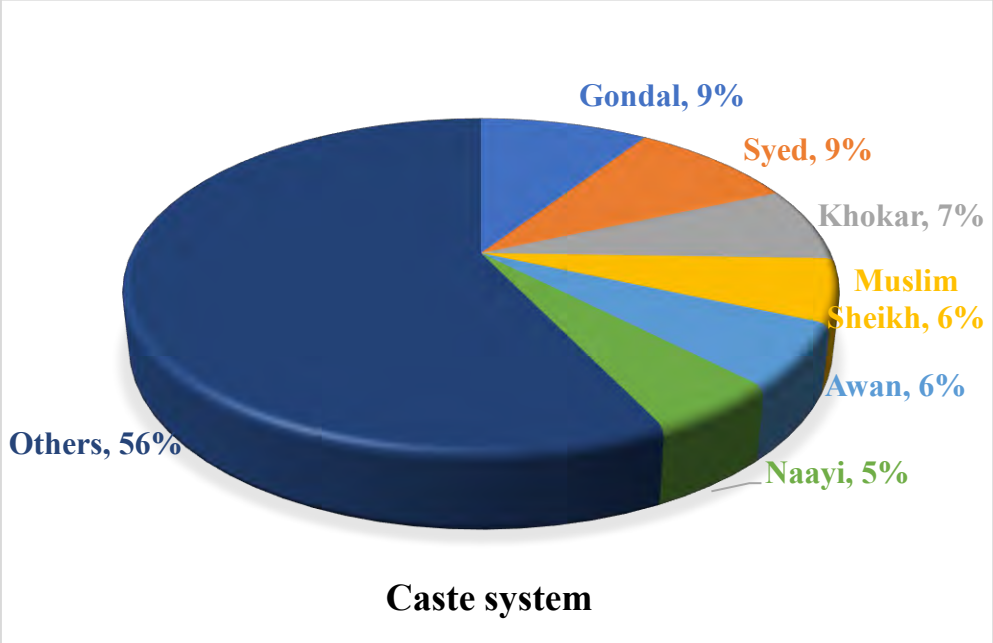


Fig.3.1.6 Distribution of subjects with respect to caste system

3.1.7 Distribution of subjects with respect to language

As the town of study was from northern Punjab, almost all the subjects had Punjabi as their mother language and only 2 subjects were Pashto speaking. The distribution of subjects with respect to language was statistically non-significant (Table 2).

3.1.8 Distribution of subjects with respect to father's education

Of 201 subjects, (n=152)76% fathers of the subjects were literate and the remaining 24% (n=49) were illiterate had no basic education at all. The distribution of subjects with respect to father education was non-significant (Table 2).

3.1.9 Distribution of subjects with respect to father's occupation

The majority of the subject's fathers were (55; 27%) were laborers and farmers (50;25%). Fathers of 26 subjects were shopkeepers by occupation and (n=22) 11% father lived abroad. Out of all, 39 subject's fathers were merged in the category of others. The distribution of subjects with respect to father occupation was statistically non-significant (Table 2: Fig.3.1.7).

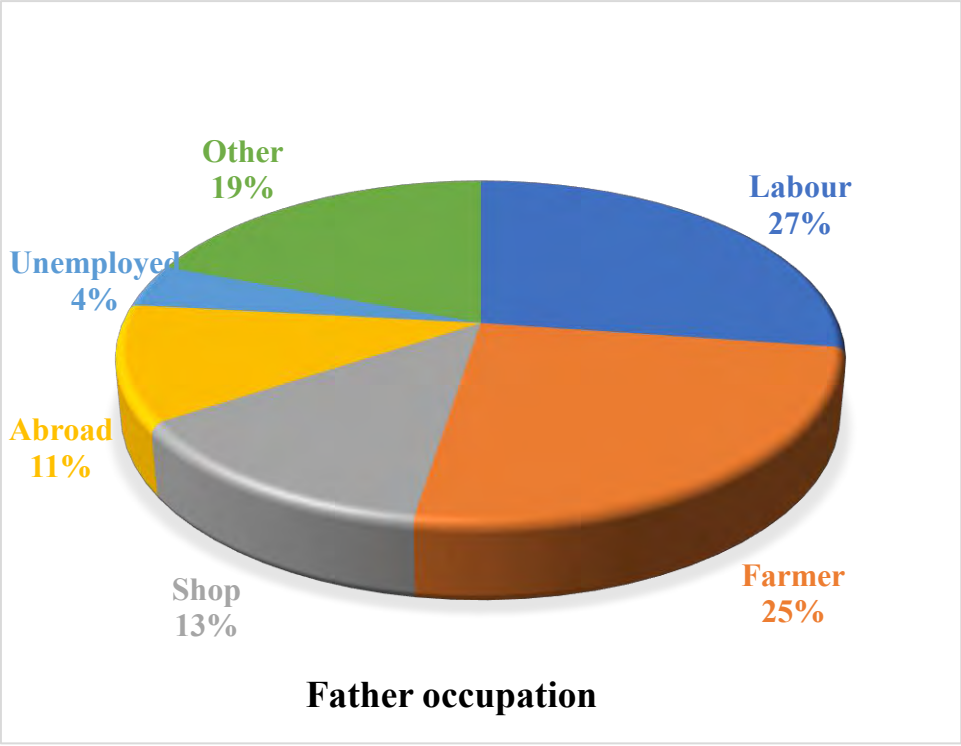


Fig.3.1.7 Distribution of subjects with respect to father's occupation

Table 2 Demographic details with respect to gender

Demography	Male		Female		Total	
	No.	%age	No.	%age	No.	%age
Origin						
Rural	76	70	33	30	109	54
Urban	52	57	40	43	92	46
Sum	128	64	73	36	201	100
			χ^2 =3.76; df =1; p=0.053; non-significant			
Age (in years)						
Up to 9	39	63	23	37	62	31
>9-19	73	65	40	35	113	56
>19-29	12	75	4	25	16	8
>29	4	40	6	60	10	5
			χ^2 =3.37; df = 3; p=0.338; non-significant			
Subject Education (≥ 5 years; n=191)						
Literate	85	62	50	36	137	72
Illiterate	38	68	18	32	56	29
			χ^2 =0.41; df = 1; p=0.521; non-significant			
Economic Status						
Poor	26	62	16	38	42	21
Lower Middle	58	68	27	32	85	42
Middle	41	61	26	39	67	33
Upper middle	3	43	4	57	7	3
			χ^2 =0.23; df = 3 p=0.510; non-significant			
Family type						
Nuclear	74	66	38	34	112	56
Extended	54	61	35	39	89	44
			χ^2 =0.62; df = 1 p=0.429; non-significant			
Caste system						
Gondal	16	84	3	16	19	9
Syed	9	50	9	50	18	9
Khokar	5	33	10	67	15	7
Muslim Sheikh	8	62	5	38	13	6
Awan	10	83	2	17	12	6
Naayi	7	64	4	36	11	5
Other	73	65	40	35	113	56

			χ^2 =12.96; df = 6 p=0.044; **significant**			
Language						
Punjabi	126	63	73	37	199	99
Pashto	2	100			2	1
			χ^2 =1.15; df = 1 p=0.283; non-significant			
Father Education						
Literate	94	62	58	38	152	76
Illiterate	34	69	15	31	49	24
			χ^2 =0.91; df = 1 p=0.339; non-significant			
Father occupation						
Labour	35	64	20	36	55	27
Farmer	34	68	16	32	50	25
Shop	17	65	9	35	26	13
Abroad	16	73	6	27	22	11
Unemployed	6	67	3	33	9	4
Other	20	51	19	49	39	19
			χ^2 =3.84; df = 5 p=0.573; non-significant			

3.2 Distribution of major and minor anomalies

A total of 201 cases with different hereditary and congenital anomalies were recruited and were classified into 4 major categories. Out of these sensorineural defects with 58% were most common followed by neurological disorders (20%), neuromuscular anomalies (16%), and visual impairments (6%). Using OMIM and ICD-10 databases, major categories were further classified into minor categories. The 'Others 'category contains anomalies with a smaller number of index cases (Table 3; Fig.3.2.1).

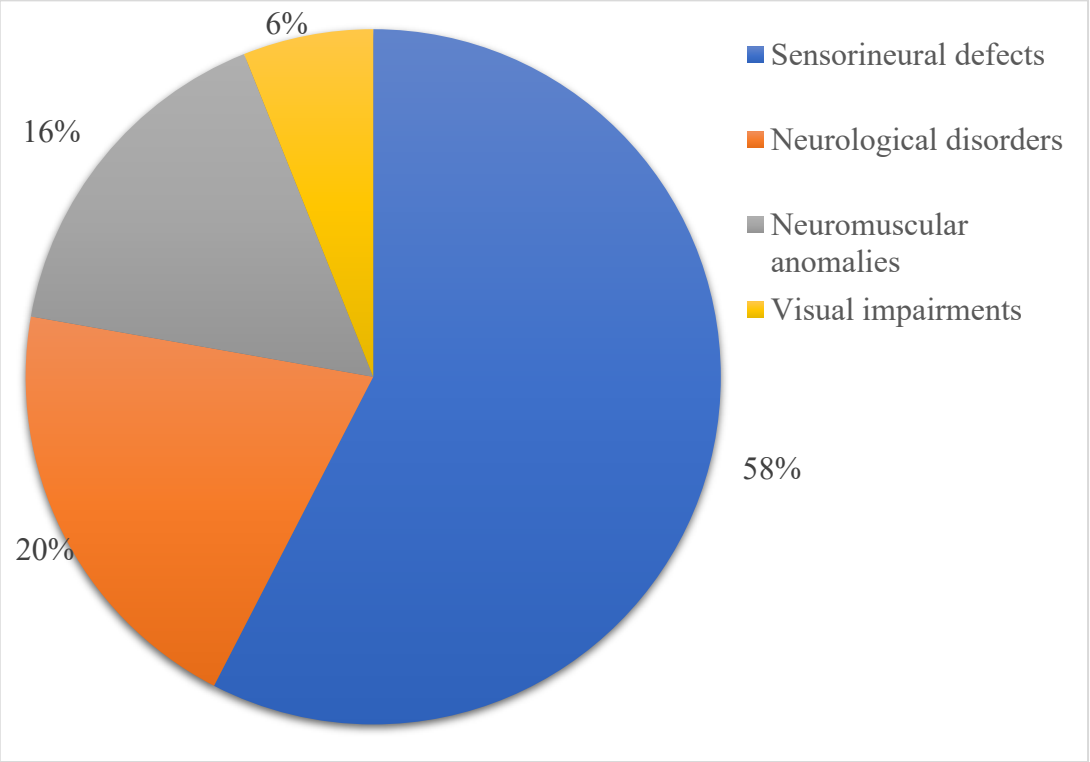


Fig. 3.2.1 Distribution of major anomalies

Table 3 Distribution of major and minor categories of congenital anomalies

Major/minor categories	Frequency	Proportion	OMIM	ICD-10
Sensorineural defects(n=115)				
Deaf and mute	113	0.982	220290	H91.3
Mute only	2	0.017		
Neurological disorders(n=41)				
Intellectual disability	35	0.853	300243	F71
Hydrocephaly	1	0.024	236600	G91.9
Microcephaly	1	0.024	156580	Q02
Autism	2	0.048	209850	F84. 0
Down's syndrome	1	0.024	190685	Q90.9
Macrocephaly	1	0.024	248000	Q75.3
Neuromuscular anomalies(n=30)				
Cerebral palsy	30	0.967	612900	G80.9
Visual Impairments(n=12)				
Retinitis pigmentosa	8	0.666	300029	H35.52
Cataract	1	0.083	610019	H26.9
blindness	3	0.25		
Others(n=3)				
Achondroplasia	1	0.5	100800	Q77.4
Club foot	1	0.5	119800	M21.541
Muscle hypotonia	1	0.032		P94.1

3.2.1 Distribution in major divisions of anomalies with respect to gender

There were four major disease divisions in the current study and all these major divisions were further analyzed with reference to gender. In sensorineural defects, 57% affected subjects were male while the remaining 43% were females. In neurological disorders, 31 (76%) and 10 (24%) were males and females, respectively. In neuromuscular anomalies, out of 33 total cases, 24 subjects (73%) were males, and 9 subjects (27%) were females. In remaining, males were more affected. The distribution of subjects with respect to their major disease division was statistically non-significant (p=0.099) (Table 4; Fig.3.2.2).

Table 4 Distribution in major divisions of anomalies with respect to gender

Major Divisions	Male		Female		Total	
	No.	%	No.	%	No.	%
Sensorineural defects	65	57	50	43	115	58
Neurological disorders	31	76	10	24	41	20
Neuromuscular anomalies	24	73	9	27	33	16
Visual impairments	8	67	4	33	12	6
Total	**128**	**64**	**73**	**36**	**201**	**100**

χ^2 =6.29; df = 3 p=0.099; non-significant

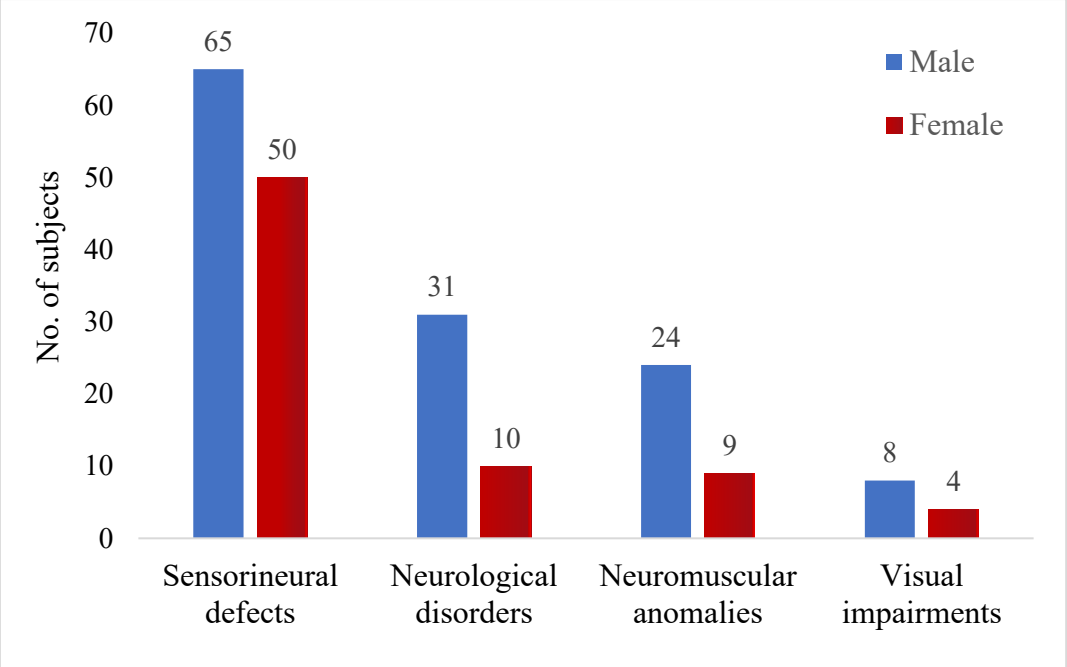


Fig. 3.2.2 Distribution in major divisions of anomalies with respect to gender

3.2.2 Distribution in major divisions of anomalies with respect to familial/sporadic nature

In both sensorineural defects and neuromuscular anomalies, 61% of the cases were sporadic in nature. While in neurological disorders, out of 41 total cases, (n=14)34% cases were familial in nature and 66%(n=27) cases were sporadic. In visual impairments, 67%(n=8) and 33% (n=4) cases were familial and sporadic respectively. The distribution with respect to familial attributes was statistically non-significant (Table 5; Fig. 3.2.3).

Table 5 Distribution in major divisions of anomalies with respect to familial/sporadic nature

Major Divisions	Familial		Sporadic		Total	
	No.	%	No.	%	No.	%
Sensorineural defects	45	39	70	61	115	58
Neurological disorders	14	34	27	66	41	20
Neuromuscular anomalies	13	39	20	61	33	16
Visual impairments	8	67	4	33	12	6
Total	**80**	**40**	**121**	**60**	**201**	**100**

χ^2 =4.19; df = 3 p=0.242; non-significant

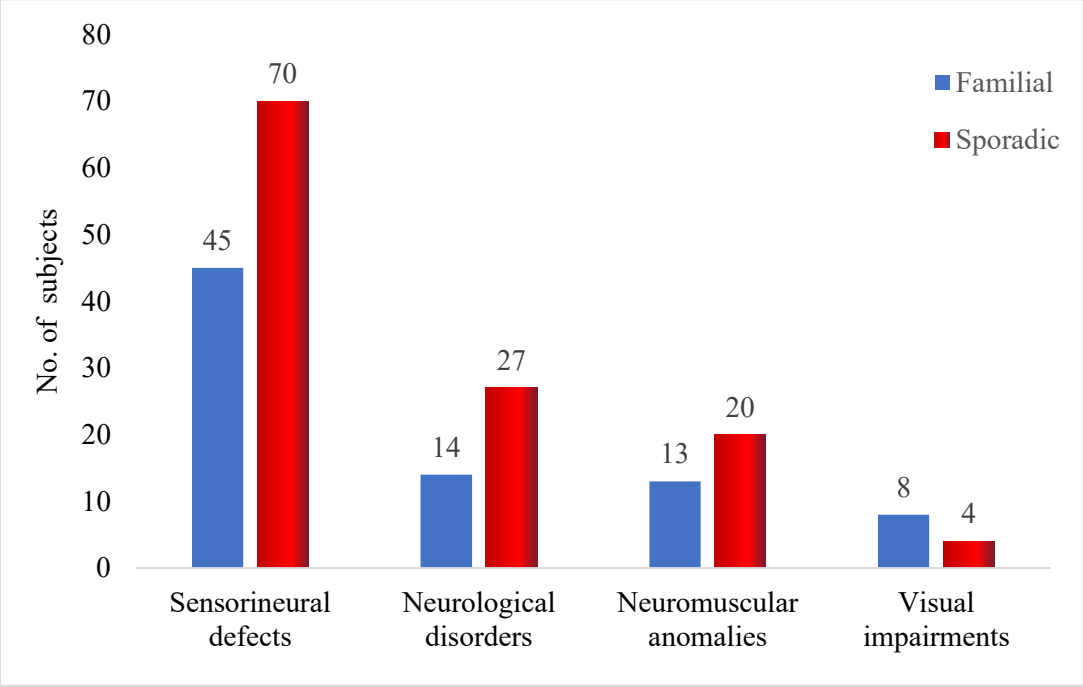


Fig. 3.2.3 Distribution in major divisions of anomalies with respect to familial/ sporadic

3.2.3 Distribution in major divisions of anomalies respect to isolated/syndromic.

In sensorineural defects,95% of affected subjects had isolated anomaly while the remaining 5% were with syndromic anomaly. In neurological disorders, 34%(n=14) subjects were syndromic while the remaining 66%(n=27) subjects had isolated anomalies. For neuromuscular anomalies and visual impairments, 9% and 2% of subjects were syndromic. The distribution with respect to isolated/syndromic was statistically highly significant (Table 6).

Table 6 Distribution in major divisions of anomalies respect to isolated/syndromic

Major Divisions	Isolated		Syndromic		Total	
	No.	%	No.	%	No.	%
Sensorineural defects	109	95	6	5	115	58
Neurological disorders	27	66	14	34	41	20
Neuromuscular anomalies	24	73	9	27	33	16
Visual impairments	10	83	2	17	12	6
Total	**170**	**85**	**31**	**15**	**201**	**100**

χ^2 =23.77; df = 3; p<0.0001; **highly significant**

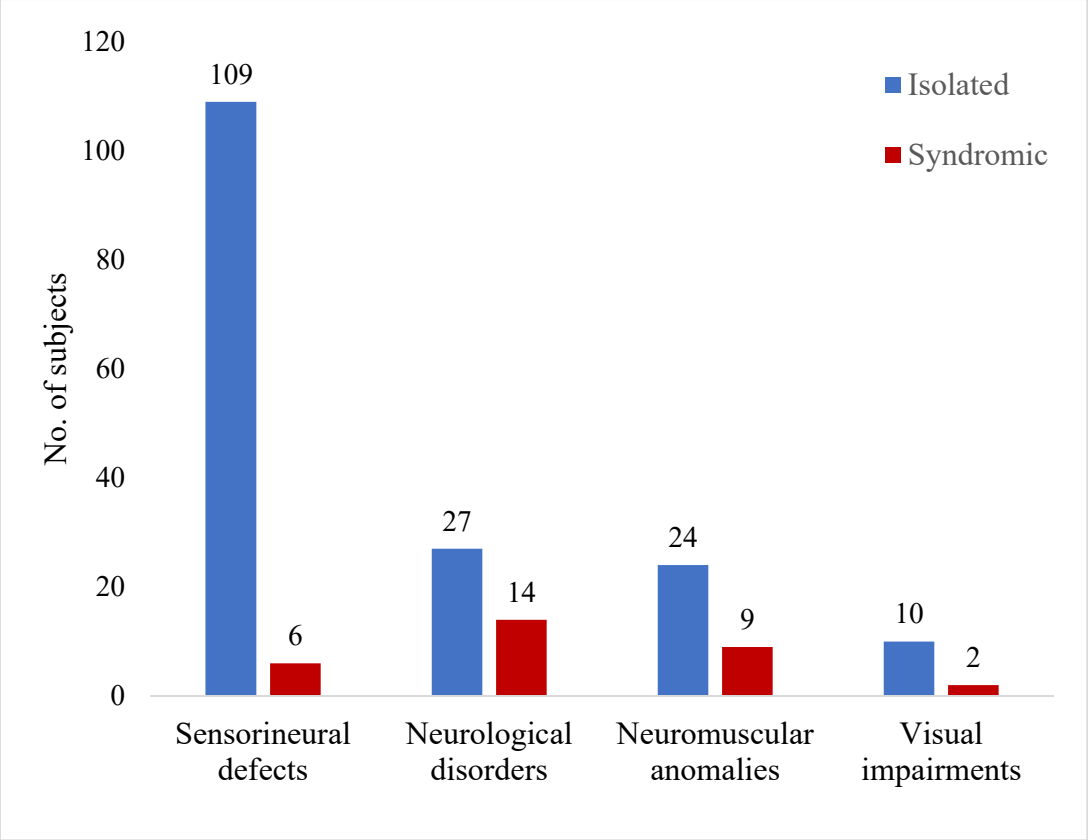


Fig.3.2.4 Distribution in major divisions of anomalies with respect to isolated/syndromic

3.2.4 Distribution in major divisions of anomalies with respect to generation with the disease

Out of the total, 80 cases were familial which were further divided based on generation with the disease. Among all familial cases, 54% (n=43) cases were found with only one generation affected and 46%(n=37) cases were found with 2 generations affected. The distribution of subjects with respect to generation with the disease was statistically non-significant (Table 7; Fig 3.2.5).

Table 7 Distribution in major divisions of anomalies with respect to generation with the disease.

Major Divisions	No. of Familial cases n=80	Generation with disease				Total	
		1		2			
		No.	%	No.	%	No.	%
Sensorineural defects	45	27	60	18	40	45	56
Neurological disorders	14	6	43	8	57	14	18
Neuromuscular anomalies	13	6	46	7	54	13	16
Visual impairments	8	4	50	4	50	8	10
Total	**80**	**43**	**54**	**37**	**46**	**80**	**100**

χ^2 =1.72; df = 3 p=0.632; non-significant

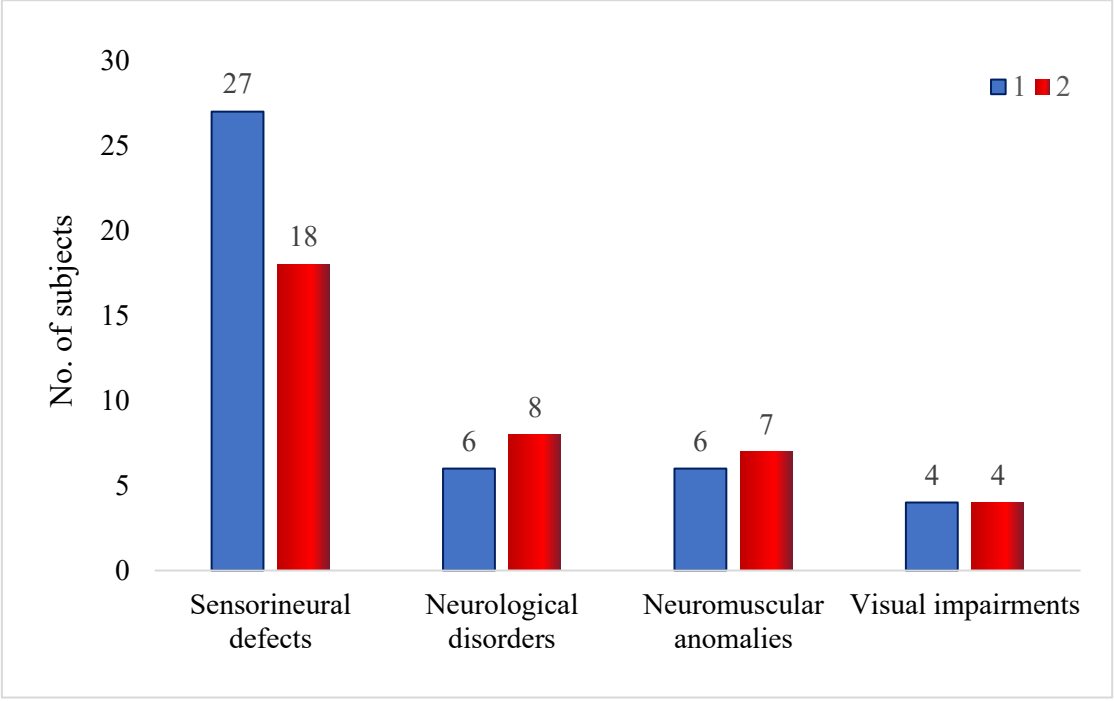


Fig. 3.2.5 Distribution in major divisions of anomalies with respect to generation with the disease

3.2.5 Distribution in major divisions of anomalies with respect to sibship with disease

Of all 80 familial cases, for sensorineural defects 38%(n=17) subjects had one sibship, while 62%(n=28) had two sibships. Similarly, for total of 14 familial cases of neurological disorders, 93% (n=13) subjects had one sibships. 88%(n=7) subjects for visual impairments had two sibships. The distribution of major divisions of anomalies with respect to sibships was statistically highly significant (Table 8).

Table 8 Distribution in major divisions of anomalies with respect to sibship with disease.

Major Divisions	No. of Familial cases n=80	Sibship of disease						Total	
		1		2		≥3			
		No.	%	No.	%	No.	%	No.	%
Sensorineural defects	45	17	38	28	62	-	-	45	56
Neurological disorders	14	13	93	-	-	1	7	14	18
Neuromuscular anomalies	13	-	-	12	92	1	8	13	16
Visual impairments	8	1	13	7	88	-	-	8	10
Total	**80**	**31**	**39**	**47**	**59**	**2**	**3**	**80**	**100**

χ^2 =32.92; df = 6 p<0.0001; **highly significant**

3.2.6 Distribution in major divisions of anomalies with respect to affected family members

The number of affected family members was 207 among eighty familial cases. A total of 64% of families had at least two affected family members. This was followed by families with three affected members 24% and with more than or equal to four affected members were found to be 13%. The distribution of a number of affected subjects in their major division was statistically highly significant (Table 9).

Table 9 Distribution in major divisions of anomalies with respect to affected family members.

Major Divisions	**No. of Familial cases n=80**	**Affected Family Member(n=207)**						**Total**	
		2		**3**		**≥4**			
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Sensorineural defects	45	30	67	12	27	3	7	45	56
Neurological disorders	14	8	57	5	36	1	7	14	18
Neuromuscular anomalies	13	11	85	1	8	1	8	13	16
Visual impairments	8	2	25	1	13	5	63	8	10
Total	**80**	**51**	**64**	**19**	**24**	**10**	**13**	**80**	**100**

χ^2 =23.56; df = 6 p=0.0006; **highly significant**

3.2.7 Distribution in major divisions of anomalies with respect to total affected persons

In all major disease divisions, a total of 328 patients were affected of which 63%(n=208) were males and 27%(n=120) were females. In sensorineural defects, 57%(n=102) and 43%(n=78) were affected males and females, respectively. This disease division has the highest number of affected persons (n=180; 33%). In neuromuscular anomalies, 76%(n=37) males and 24% (n=12) females were affected. Neurological disorder and visual impairments were also more common in males than females. The distribution of subjects with respect to affected persons was statistically significant (Table 10; Fig.3.2.6).

Table 10 Distribution in major divisions of anomalies with respect to affected persons

Major Divisions	**Male**		**Female**	
	No.	**%**	**No.**	**%**
Sensorineural Defects	102	57	78	43
Neurological Disorders	44	71	18	29
Neuromuscular Anomalies	37	76	12	24
Visual Impairments	25	68	12	32
Total	**208**	**63**	**120**	**27**

χ^2 =8.42; df = 3 p=0.0380; **significant**

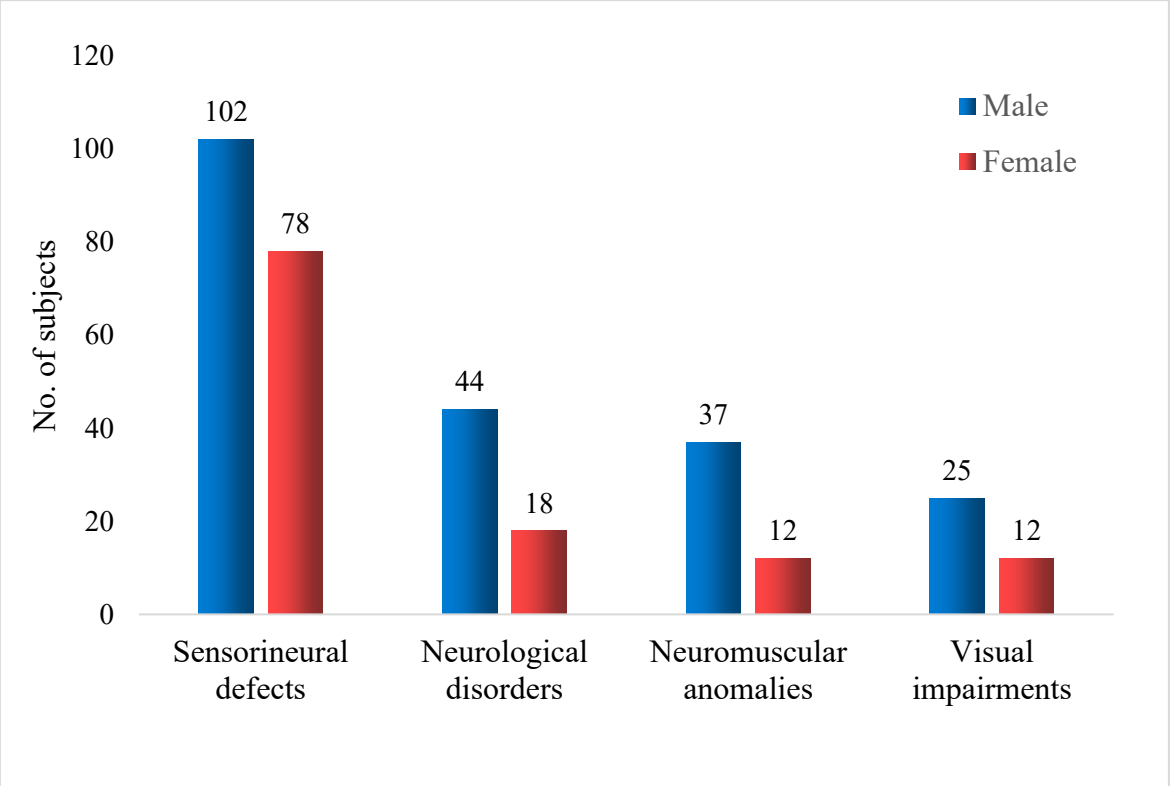


Fig. 3.2.6 Distribution in major division of anomalies with respect to affected person

3.2.8 Distribution in major divisions of anomalies with respect to parity of the index

All the major disease divisions were analyzed with respect to the parity of the index persons. Out of 201 cases, 81(40%) subjects were the first kid in their families. In sensorineural defects 46(40%) subjects were the first borne of their families. For neurological disorders, 15 subjects had the first parity number in their families while 6 had second. In neuromuscular anomalies, 14(42%) subjects had first parity. In remaining cases, the parity of the index subject was 2nd (n=44), 3rd (n=26), 4th (n=22), 5th (n=15), 6^{th} and greater than 6th (n=13). The distribution of major categories with respect to parity was statistically non-significant (Table 11).

Table 11 Distribution in major divisions of anomalies with respect to parity of the index

Major Categories	1st		2nd		3rd		4th		5th		≥6th		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sensorineural Defects	46	40	30	26	13	11	12	10	10	9	4	3	115	58
Neurological Disorders	15	37	6	15	5	12	7	17	2	5	6	15	41	20
Neuromuscular Anomalies	14	42	6	18	5	15	3	9	3	9	2	6	33	16
Visual Impairments	6	50	2	17	3	25	.	.	.	.	1	8	12	6
Total	**81**	**40**	**44**	**22**	**26**	**13**	**22**	**11**	**15**	**7**	**13**	**6**	**201**	**100**

χ^2 =14.81; df = 15 p=0.465; non-significant

3.3 Distribution of risk factors with respect to gender

3.3.1 Distribution of subjects with respect parental marriage type

Among 201 of total cases, consanguineous union was 61% more common for male index parents as compared to females where only 39% parents had consanguineous marriage type. The distribution of gender-wise data with respect to parental marriage type was statistically non-significant (Table 12).

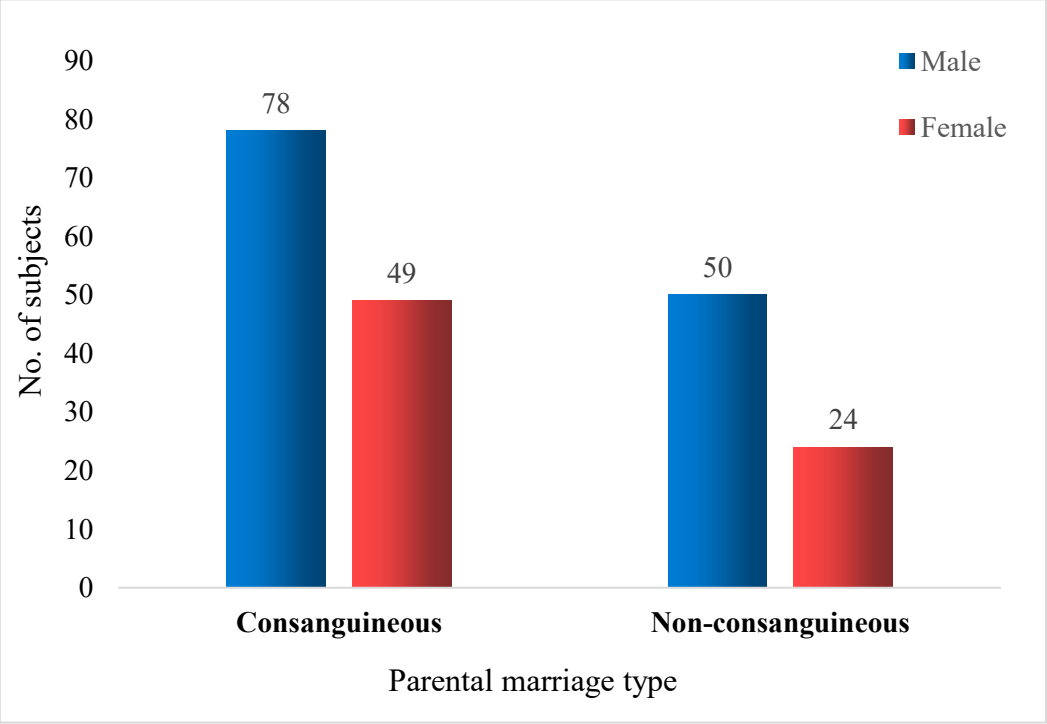


Fig.3.3.1 Distribution of subjects with respect parental marriage type

3.3.2 Distribution of subjects with respect to maternal age

Maternal age at time of subject birth can be a risk factor for congenital anomalies. Maternal ages were divided into four categories. For 37%(n=74) index subjects, maternal age ranged from 25 to 29 years, while for 34%(n=69) cases maternal age was less than 25 years. Only for 10% cases, maternal age was greater than 35 years. The distribution of gender-wise data with respect to maternal age was statistically non-significant (Fig. 3.3.2; Table 12).

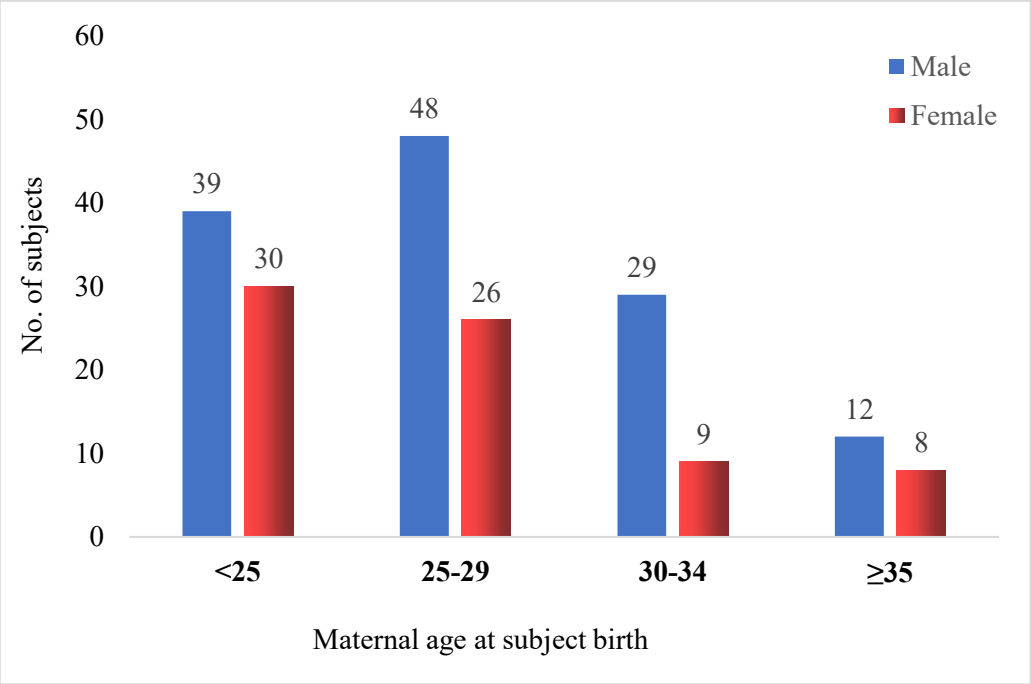


Fig.3.3.2 Distribution of subjects with respect to maternal age

3.3.3 Distribution of subjects with respect to mode of delivery

Based on mode of delivery, index cases were categorized into two groups. Index subjects born through normal delivery were in major presentation of 83% and the distribution of gender-wise data with respect to mode of delivery was statistically highly significant (Fig. 3.3.3; Table 12).

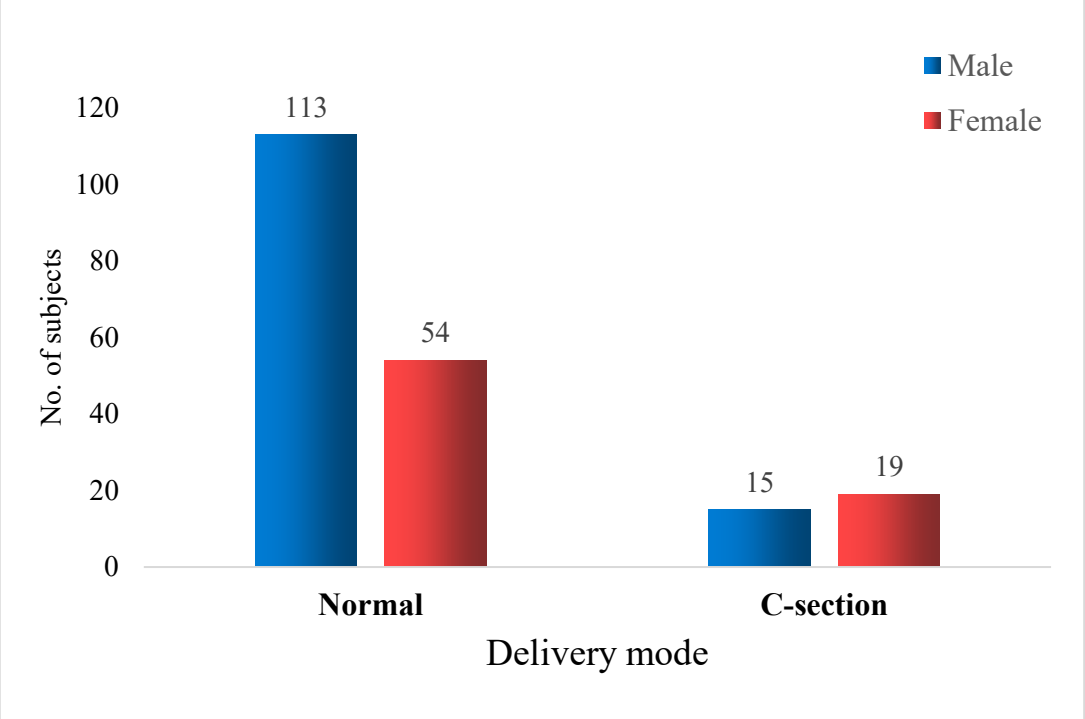


Fig.3.3.3 Distribution of subjects with respect to delivery mode

3.3.4 Distribution of subjects with respect to delivery spot

With respect to delivery spots, index cases were split into two groups. Home-based delivery showed a high percentage of 74 % and the distribution of gender-wise data with respect to delivery spot was statistically significant (Fig. 3.3.4; Table 12).

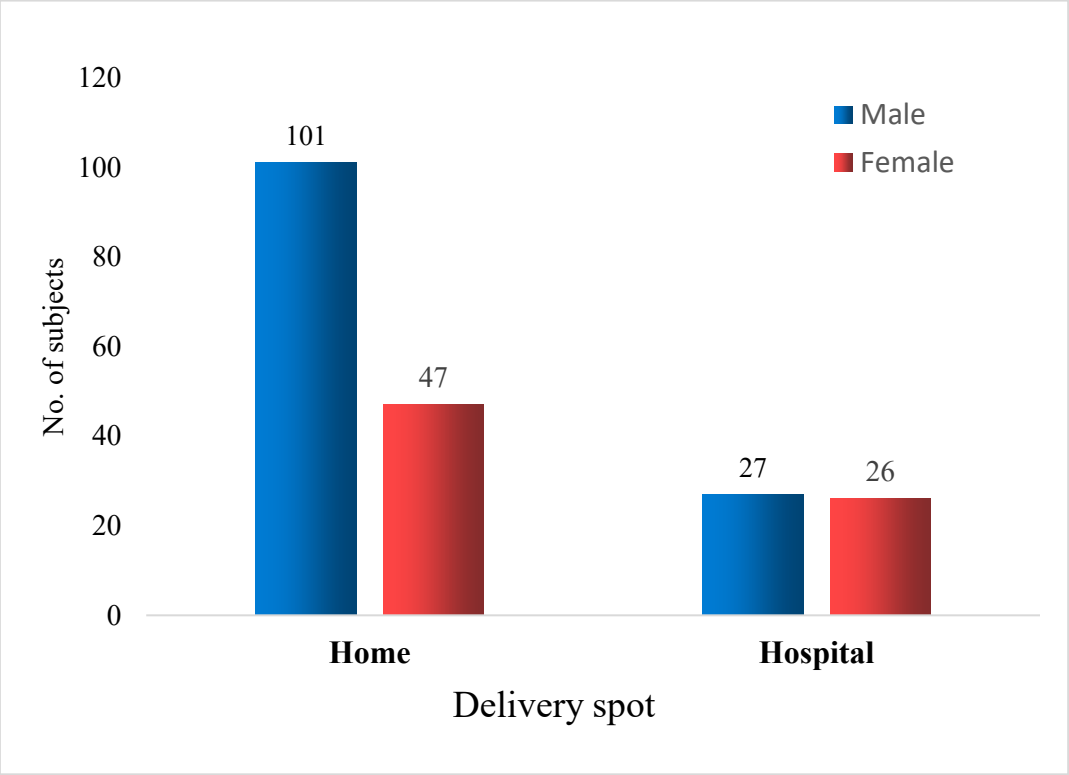


Fig.3.3.4 Distribution of subjects with respect delivery spot

3.3.5 Distribution of subjects with respect to family history

Of all cases studied, only 40% of subjects had prior family history of same or related anomaly, while 60% cases had no family history of the disorder. The distribution of gender-wise data with respect to family history was statistically non-significant (Fig.3.3.5; Table 12).

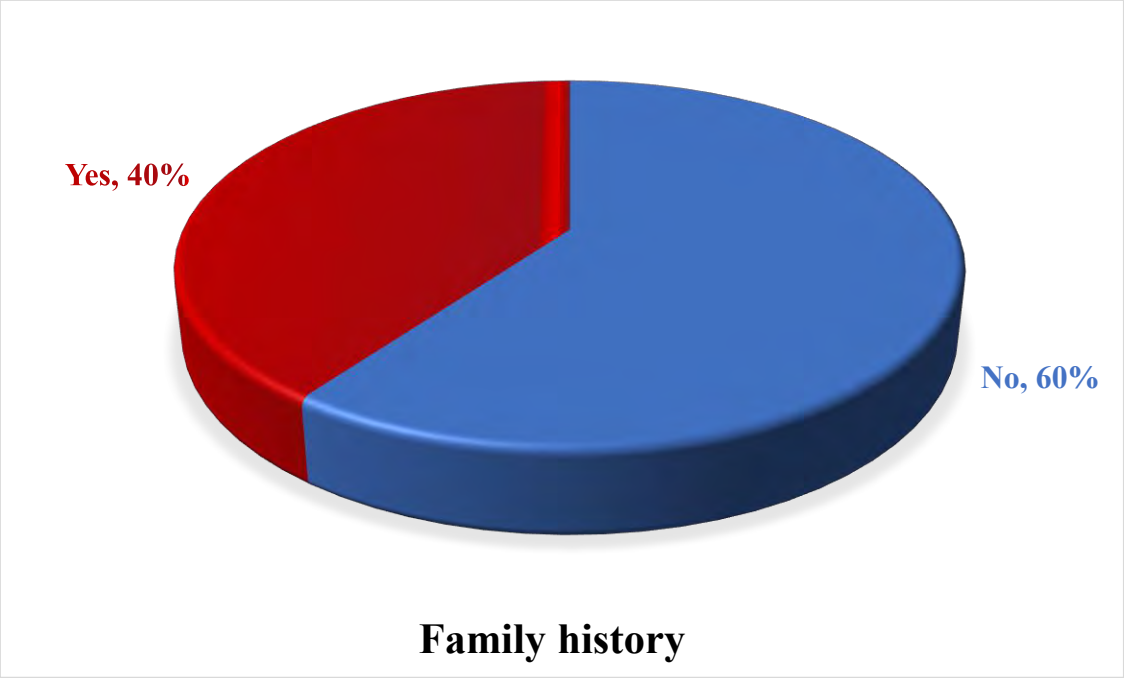


Fig.3.3.5 Distribution of subjects with respect to family history

Table 12 Distribution of risk factors with respect to gender

Risk Factors	Male		Female		Total	
	No.	%age	No.	%age	No.	%age
Parental marriage types						
Consanguineous	78	61	49	39	127	63
Non-consanguineous	50	68	24	32	74	37
Sum	128	64	73	36	201	100
			χ^2 =0.765; df = 2 p=0.682; non-significant			
Maternal Age						
<25	39	57	30	43	69	34
25-29	48	65	26	35	74	37
30-34	29	76	9	24	38	19
≥35	12	60	8	40	20	10
			χ^2 =4.31; df = 3 p=0.231; non-significant			
Delivery Mode						
Normal	113	68	54	32	167	83
C-section	15	44	19	56	34	17
			χ^2 =6.77; df = 1 p=0.009; **highly significant**			
Delivery Spot						
Home	101	68	47	32	148	74
Hospital	27	51	26	49	53	26
			χ^2 =5.05; df = 1 p=0.025; **significant**			
Family History						
Yes	49	65	31	35	80	40
No	79	61	42	39	121	60
			χ^2 =0.341; df = 1 p=0.560; non-significant			

3.4 Parental parameters

3.4.1 Parental consanguinity

To find out the marriage types among the parents of index cases, data was analyzed, and it indicated that there were 63% (n=127) cases when consanguineous marriage type was found whereas non-consanguineous marriages were found in only 37% (n=74) cases (Fig. 3.4.1).

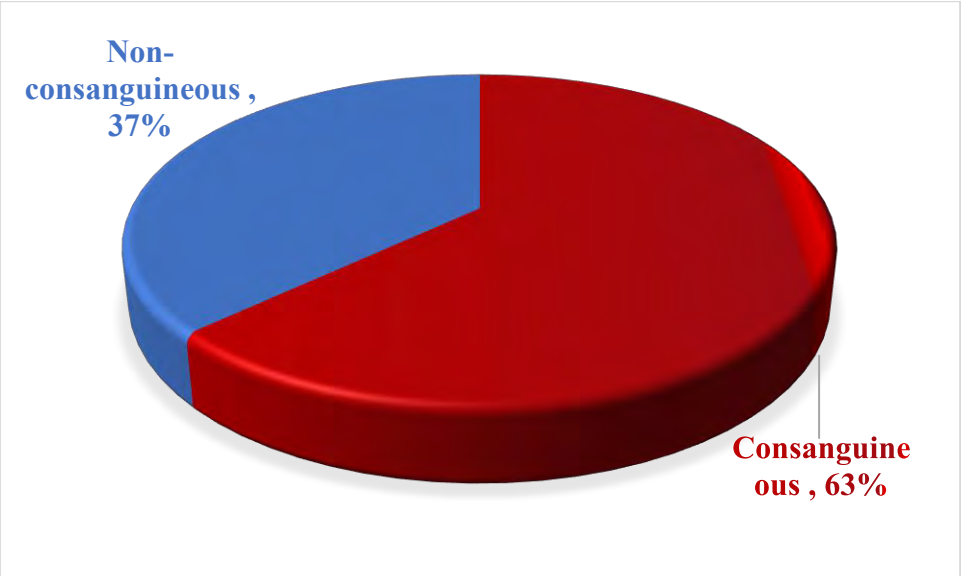


Fig.3.4.1 Parental consanguinity

3.4.2 Distribution of anomalies with respect to consanguinity

Consanguineous marriages were more common in both sensorineural defects and neurological disorders. For neuromuscular anomalies less(n=20) consanguineous parental marriage union was observed whereas for visual impairments, the results were same as first two categories, i.e. consanguineous marriages more common (Fig.3.4.2).

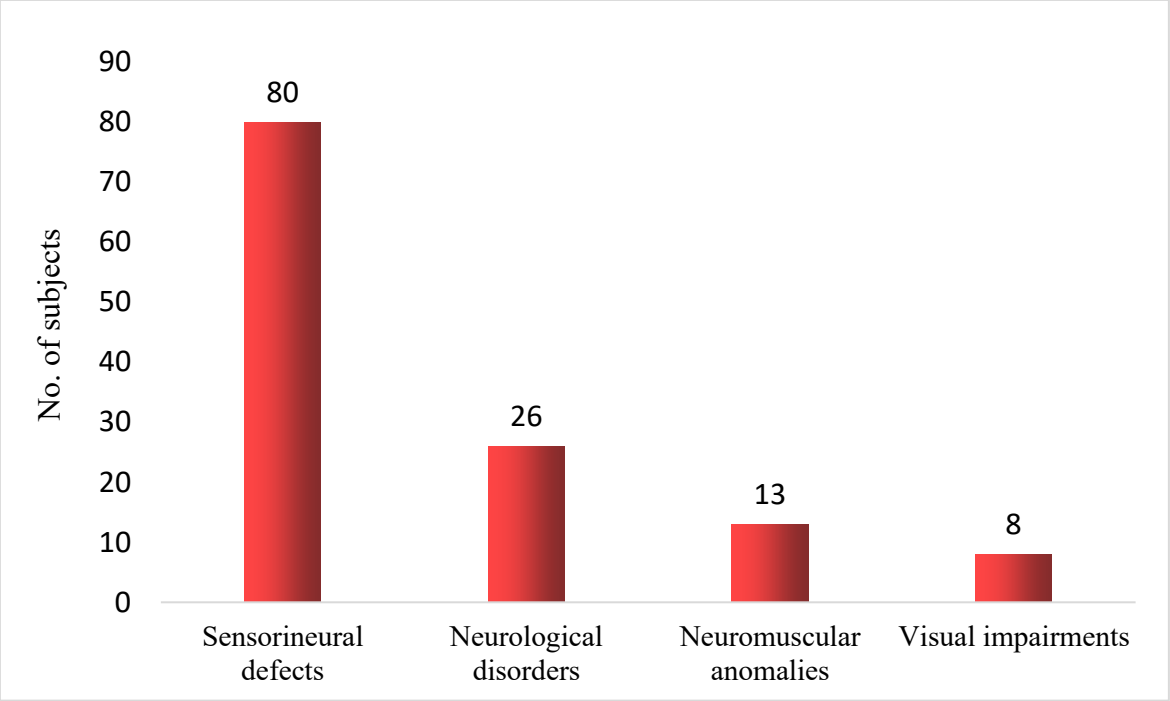


Fig. 3.4.2 Distribution of anomalies with respect to consanguinity

3.4.3 Distribution of parental marriage types with respect to familial/sporadic nature

Familial consanguineous cases (n=62) were more common as compared to familial non-consanguineous cases (n=18). Sporadic consanguineous cases were also more frequent with (n=65) as compared to sporadic non-consanguineous cases (n=56). The distribution of parental marriage type with respect to familial/sporadic nature was statistically highly significant (Table 13).

Table 13 Distribution of parental marriage types with respect to familial/sporadic nature

Parental Marriage type	**Familial**		**Sporadic**		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Consanguineous	62	49	65	51	127	63
Non-consanguineous	18	24	56	76	74	37
Total	**80**	**40**	**121**	**60**	**201**	**100**

χ^2 =11.71; df = 1 p=0.006; **highly significant**

3.4.4 Average parental age at the birth of subjects

The data was analyzed for various anomaly types, and the average ages of both fathers and mothers were calculated accordingly. The findings revealed (Table 14) that visual impairments and neuromuscular anomalies had the highest average paternal and maternal ages, with 35.3 and 28.1 years, respectively. For neurological disorders, the average paternal age was 32.6 years, and the average maternal age was 27.7 years. Below is a detailed explanation of each anomaly type.

Table 14 Distribution of average parental age with respect to major divisions

Major Divisions	**No. of cases**	**Average parental age**	
		Paternal age	**Maternal age**
Sensorineural Defects	115	31.1	26.5
Neurological Disorders	41	32.6	27.7
Neuromuscular Anomalies	33	32.2	28.1
Visual Impairments	12	35.3	25.3
Average age		31.9	26.9

3.5 Representative hereditary disorders

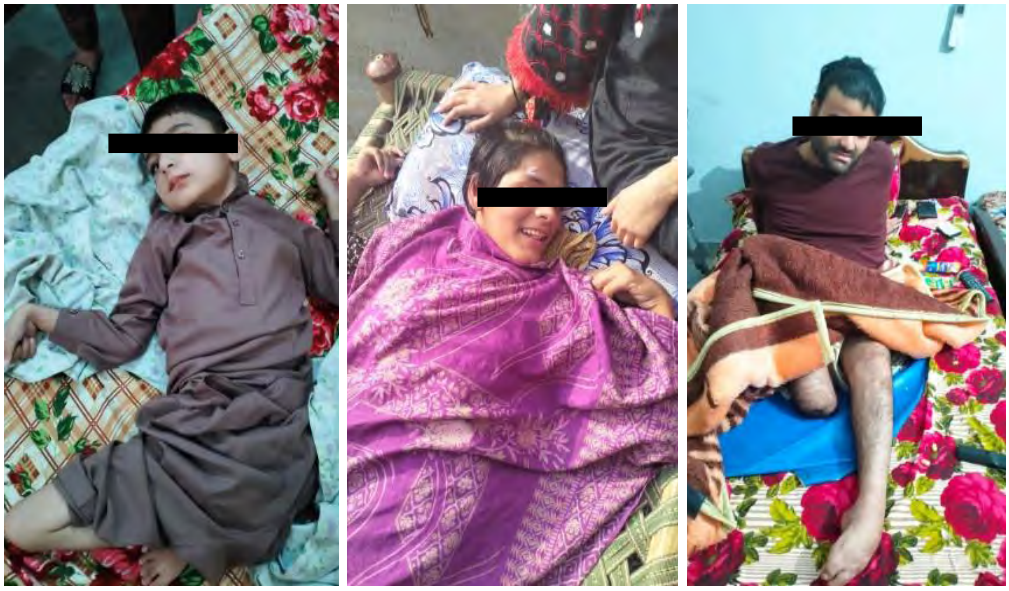
Some representative phenotypes encountered during the study are shown in figures 3.5.1-3.5.2 and pedigrees of some of the familial cases with 3 or more affected members are shown in figures 3.6.1-3.6.2.

3.5.1 Less severe phenotypic manifestation

A. Down's syndrome B. Achondroplasia C. Blindness

Fig. 3.5.1 A: Down syndrome (presence of extra chromosome no 21), B: Achondroplasia (unusually short stature), C: Blindness

3.5.2 More severe phenotypic manifestation



A. Cerebral palsy B. Cerebral palsy C. Muscle hypotonia

Fig. 3.5.2 A & B: Cerebral Palsy, C: Muscle hypotonia

3.6 Pedigrees of familial cases

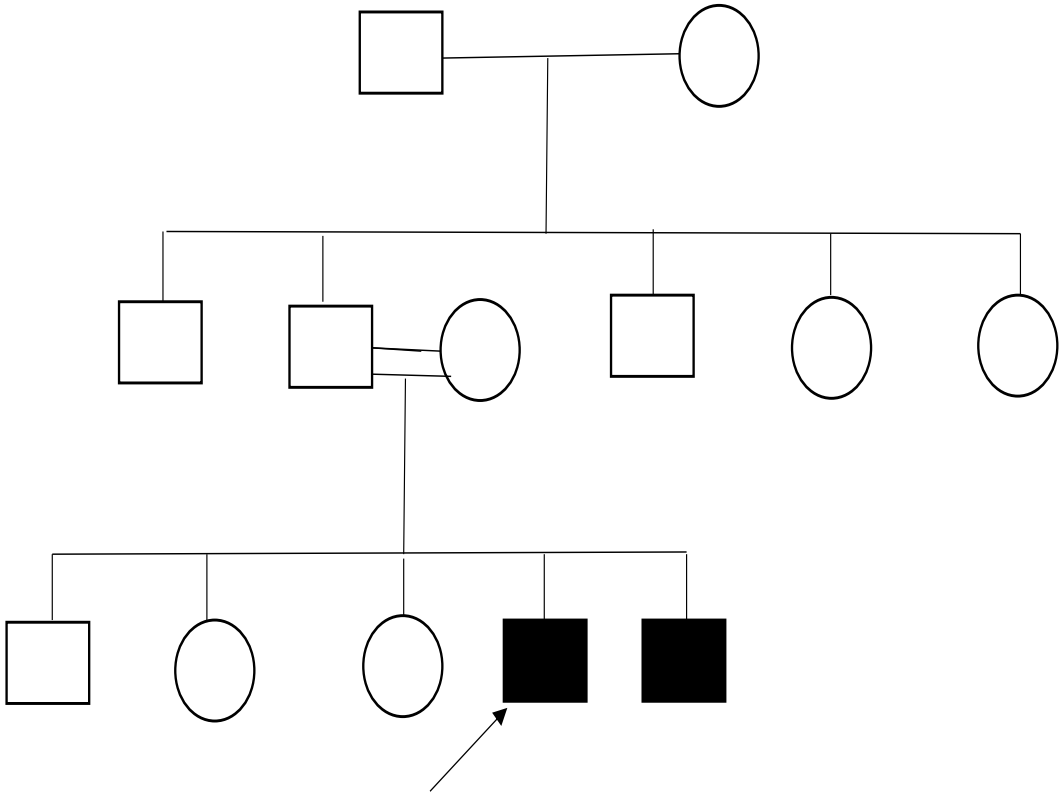


Fig. 3.6.1 A three generation pedigree of a family with mental retardation

*arrow indicates the index case

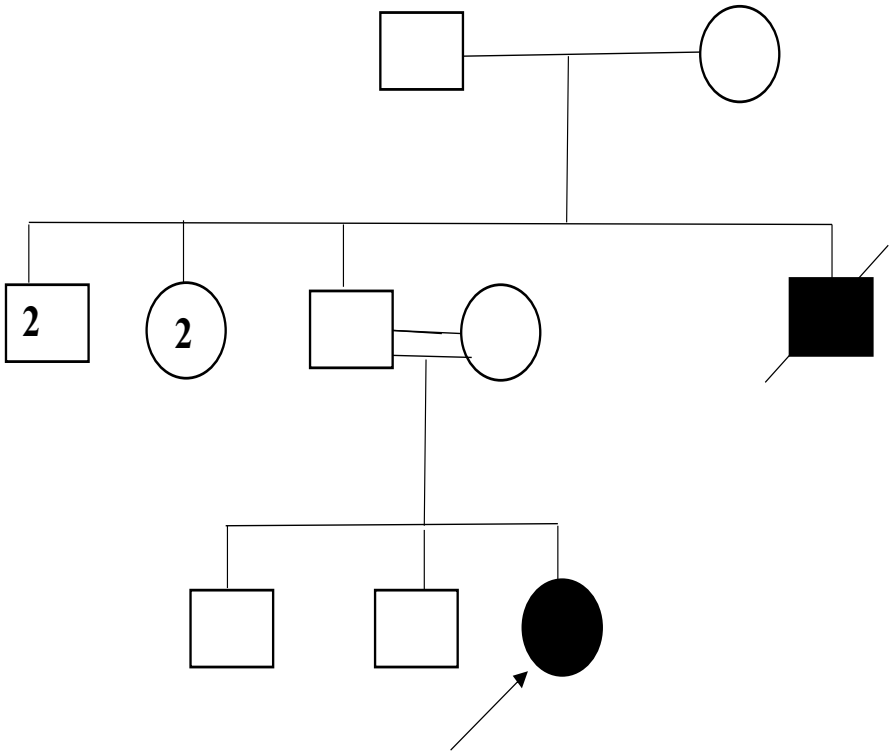


Fig 3.6.2 A three generation pedigree of a family with Cerebral Palsy (CP)

*arrow indicates the index case

Genetic disorders continue to be a significant concern for geneticists and a source of anxiety for expectant women. Individuals affected by genetic disorders face various challenges in their daily lives, necessitating extra parental care and becoming a financial burden on their families and society. Job opportunities are often limited for them compared to unaffected individuals, and they also encounter socio-psychological barriers, hindering their full integration into society.

Managing genetic disorders poses a formidable challenge for healthcare systems due to the need for lifelong medical attention, costly supportive and symptomatic therapies, and specialized care. Conducting studies to explore the range of genetic disorders is crucial for establishing baseline rates, monitoring changes over time, and potentially uncovering their underlying causes. These studies also play a vital role in developing and evaluating prenatal screening for congenital anomalies, especially in high-risk populations. Understanding the prevalence of genetic disorders within our populations would aid in the development of more effective healthcare systems and in predicting potential future increases or decreases in the burden of congenital anomalies. Globally, congenital anomalies have been reported to account for 20% of infant mortality (Chung et al., 2013).

The main objective of this study is to estimate the prevalence and pattern of genetic anomalies in the Mandi Bhauddin region, as no previous research has explored the state and pattern of congenital anomalies in this area. Furthermore, the study aims to assess the prevalence of congenital malformations in this region's population and identify the risk factors associated with these defects. By addressing certain risk factors such as a high rate of consanguinity, exposure to radiation and teratogens, advanced maternal age, malnutrition,

and maternal diseases, it is possible to reduce the occurrence of deformities. To achieve this, screening approaches like determining maternal blood markers, using ultrasonography, performing amniocentesis, and collecting chorionic villus samples can be employed to detect and manage high-risk pregnancies. Throughout the investigation, some interesting associations between genetic anomalies and various factors have been discovered.

According to current study's findings, most congenital anomalies observed were related to sensorineural defects, accounting for 57% of cases. Neurological disorders accounted for 20% of cases, followed by neuromuscular anomalies at 16%, and visual impairments at 6%.

All individuals included in the study required assistance from their family members for necessities and daily tasks, indicating that they were not self-sufficient. Identifying these patients was relatively easy due to their recognizable identities within the neighborhood, making it convenient to approach them for the study. Regarding subjects with sensorineural/ear defects, they were found to be attending special schools. As a result, approached their parents through the school administration to involve them in this study.

Congenital hearing loss is particularly prominent in children, and it can be caused by environmental and prenatal factors, as highlighted by Korver et al., (2017). Globally, the prevalence of congenital hearing loss is reported to be around 1-2 per 1000 live births, according to Salvago et al., (2013). Despite its significant impact on public health, population-based studies on congenital hearing loss are not common in literature.

Notably, the present study found that sensorineural/ear defects were the most common congenital anomaly, constituting 58% (n=115) of all anomalies observed. Within the various minor groups of major congenital anomalies, the deaf and mute group emerged as the most dominant in terms of the number of cases. On a broader scale, the worldwide

prevalence of hearing defects was reported as 1.4% in children under 15 years of age, 10% in females over 15 years, and 12% in males over 15 years in 2008, as shown in the study conducted by Stevens et al., (2013).

The second most prevalent category of anomalies identified in the study was neurological disorders, accounting for 20% (n=41) of all anomalies. Within the neurological disorders, six minor categories were further classified. Among these, intellectual disability (ID) was the most common, constituting 85% (n=35) of cases. The other categories included Autism (5%), hydrocephalus (2%), Down's syndrome (2%), microcephaly (2%), and macrocephaly (2%). The findings of this study are consistent with the results reported by Taye et al., (2019), Zahra et al., (2016), and Ameen et al., (2018), all of whom also observed a high prevalence of neurological disorders in their respective studies.

The high prevalence of intellectual disability in the study area may be attributed to micronutrient deficiencies like folic acid, as mentioned by (Penchaszadeh, 2002). Adequate consumption of folic acid (400 micrograms) by women capable of bearing pregnancy are recommended to mitigate such risks. Developing countries bear a significant burden of neurological disorders, which may be influenced by factors like asphyxia, prematurity, neonatal infections, CNS infections, and consanguinity, as suggested by Chand et al., (2021). It's worth noting that certain anomalies, such as those in the neurological category, may be more readily detected at birth, leading to more accurate and careful recording, which could contribute to their seemingly higher percentage, as proposed by Tomatir et al., (2009).

In the present study, neuromuscular anomalies were identified as the third most prevalent congenital and hereditary malformations. These disorders accounted for 16% (n=33) of all anomalies observed. The neuromuscular disorders were further categorized into a single minor group, which was cerebral palsy. This pattern is consistent with a

previous study conducted in Sialkot, Pakistan, which also found a high incidence of cerebral palsy after limb defects (Bhatti et al., 2019). Another study in the Hazara district of Pakistan (Bibi et al., 2022) also reported a high prevalence of cerebral palsy.

Neuromuscular disorders encompass a diverse group of diseases primarily affecting the peripheral nerve, lower motor neuron, neuromuscular junction, or muscles, as explained by Santos et al., (2013). The present study attributed cerebral palsy cases to factors such as birth asphyxia, meningitis, fits, hypoxic ischemia, and traumas occurring during or shortly after birth. Interestingly, almost all cases of cerebral palsy were associated with other types of disorders, including developmental delay, epilepsy, visual defects, sensorineural defects, microcephaly, and intellectual disability as noted by Gulati and Sondhi (2017).

When analyzing the 201 index cases based on familial and sporadic nature, it was found that out of the total cases, 121 were sporadic, and only 80 were familial. In the case of sensorineural defects, the majority of cases were sporadic (61%), while familial cases constituted only 39%. This contrasts with the findings of Zahra et al. (2016), whose study reported the highest number of familial cases (72%) in the sensorineural/ear defects category and 28% sporadic cases. Regarding neurological disorders, a high number of sporadic cases (n=27) and familial cases (n=14) were reported, which is consistent with the findings of Zahra et al. (2016), who also observed a high number of sporadic cases in this category.

The sporadic occurrence of anomalies in the study could be attributed to non-genetic factors such as environmental influences, including poor maternal nutrition, exposure to pesticides and radiation during pregnancy, and maternal illnesses, as suggested by Harris et al., (2017). Another potential reason could be inadequate folic acid intake during the first trimester of pregnancy, which has been associated with an increased risk of congenital anomalies. The second factor contributing to sporadic anomalies may be traumas

experienced immediately after childbirth, such as meningitis, asphyxia, sepsis, fits, hypoxic ischemia, and poor antenatal care, as mentioned by Gulati and Sondhi (2017). Moreover, socio-cultural norms in the region could also play a role as people may be hesitant to disclose personal information, including consanguinity, pedigree information, and the number of family members.

Regarding the gender distribution of affected individuals in the study, it was found that males were more affected than females, with a ratio of 64% males to 36% females. This finding aligns with various other epidemiological studies, both nationally and internationally. Studies conducted in Sialkot and Hazara, Pakistan, as well as in India, Turkey, and other regions, have reported higher ratios of affected males than females in cases of congenital anomalies.

For instance, Paton et al., (2010), Sarkar et al., (2013) in India, Tomatir et al., (2009) in Turkey, Zahra et al., (2016), Ochoga et al., (2018), and Baruah et al., (2019) all found a higher percentage of affected males compared to females. Hemonta et al.'s study in Assam, India, also reported a similar trend with a higher ratio of affected males compared to females. Therefore, the present study's findings of a higher prevalence of congenital anomalies among males are consistent with previous research conducted both locally and globally.

The low ratio of female subjects in the present study can be attributed to socio-cultural norms prevailing in Pakistani society, especially in rural areas. Proper consent is often required to approach female subjects, and women may be restricted in their mobility and accessibility, making it difficult to find female subjects in public areas where most of the male subjects were ascertained.

On the other hand, the high ratio of affected males in the study may have multiple reasons. One potential explanation is that females may be afflicted with more severe

congenital anomalies and may not survive to be born with signs of life, as reported by Sarkar et al., (2013). Additionally, some genetic disorders, especially recessive disorders, require only one copy of the defective gene to be expressed in males, while females require two copies of the defective gene for the expression of such disorders.

The study also explored the role of parental factors in the development of birth defects. Consanguinity between parents and average maternal and paternal age were considered as parental attributes in this study. Consanguinity has been shown in many studies to be associated with a higher rate of birth defects. Moreover, maternal and paternal age have been linked to various fetal anomalies. Overall, understanding the influence of parental factors on the development of congenital and hereditary anomalies is crucial for identifying risk factors and implementing appropriate preventive measures in the future.

In the present study, it was observed that consanguineous marriages were more common, with 63% (n=127) of the 201 cases of congenital and hereditary anomalies having parents who were related by blood. Non-consanguineous marriages accounted for 37% (n=74) of the cases. The percentage of consanguinity in this study was higher than what was found in previous studies conducted in Sialkot (17%) and Kurram agency (55.3%). A study conducted in Turkey by Tomatir et al., (2009) reported 14.3% parental consanguinity, while our finding is more in line with the study by Gul et al., (2021), which reported 68% parental consanguinity.

Within the various types of anomalies, the highest percentage of parental consanguinity was observed in sensorineural defects (70%), while the lowest percentage was observed in neuromuscular anomalies (39%). The findings of this study are consistent with the work of Shawky and Sadik (2011), who reported that consanguinity played a significant role in the prevalence of congenital anomalies. Similarly, Riaz et al.'s study in the Rahim

Yar Khan District of Pakistan also found an association between consanguinity and the prevalence of congenital anomalies. In other regions, such as Bradford, a study by Corry (2014) revealed that 30% of major childhood disabilities were linked to the Pakistani community, where consanguinity is more common. This community accounted for 5% of all births, and the higher ratio of disabilities was attributed to the higher prevalence of consanguineous marriages in this community. Overall, the high percentage of consanguinity observed in the present study underscores the importance of considering this factor in understanding the occurrence of congenital and hereditary anomalies in the population.

The study conducted by Akram (2008) revealed that more than 80% of parents in Pakistan are first cousins, while 7% are blood relatives, 6% belong to the same caste, and only 4% are outsider marriages. This high prevalence of consanguinity in Pakistan is a notable factor to consider when studying the occurrence of congenital and hereditary anomalies in the population. In Oman, a study conducted by Rajab et al., (2014) found that parental consanguinity was significantly associated with mortality in newborns with any type of congenital anomalies. Moreover, parental consanguinity was also significantly linked to intellectual disability in children. Similarly, in Egypt, a study on the prevalence of congenital anomalies carried out by Shawky and Sadik (2011) reported that 46% of the studied population had prenatal consanguinity, which is higher than the normal prevalence of 39% in the general population. Consanguineous marriages are recognized as a common practice in the Middle East, and they play a significant role in the development of autosomal recessive or sex-linked recessive diseases in the offspring.

Overall, the high prevalence of consanguineous marriages in the region is an important contributing factor to the occurrence of congenital and hereditary anomalies. Understanding the impact of parental consanguinity is essential for addressing the risk of such anomalies and implementing appropriate preventive measures.

The study conducted in India by Sarkar et al., (2013) found that the prevalence of congenitally malformed babies was higher when born out of consanguineous marriages. The high consanguinity rate was attributed to the preference of many families for marriage among first cousins, which is done to preserve family structure, links, and to provide social, economic, and cultural benefits. There is a common belief that family marriages, including consanguineous marriages, are less likely to end in divorce, as reported by Shawky et al., (2013).

Research indicates that certain disorders, such as muscular disorders, mental retardation, blindness, diabetes, and deafness, are more likely to be inherited in children born to first cousins than to unrelated parents, as mentioned by Baig et al., (2008). In the study, maternal and paternal ages were also considered for each case. The average paternal age in each case was 32 years, while the average maternal age was 27 years. In cases of ear defects, the average paternal and maternal ages were 31 and 27 years, respectively. In cases of neurological disorders, the average paternal and maternal ages were 33 and 38 years, respectively. This information may offer valuable insights into the potential influence of parental age on the occurrence of congenital and hereditary anomalies.

In this study, 201 cases were analyzed based on the socio-economic status of families, and it was found that the majority of subjects belonged to the lower middle category, accounting for 42% (n=85) of cases. The middle category followed closely at 33% (n=67), while the poor category contributed 21% (n=42) of cases. Only a small percentage of cases, 3% (n=7), were from the high economic status category. This distribution is in line with the findings of the study conducted by Zahra et al., (2016) where most cases also belonged to the low-middle category (n=113), and only a few were from the poor/low category (n=22). However, it contrasts with the results of the study by Taye et al., (2019),

where most families fell into the middle-income category (49%), followed by low-income families (43%).

The study also analyzed the index cases based on age groups, and it was observed that most of the cases fell into the age group >9-19 years (n=113). The age group up to 9 years had the second highest representation with 62 cases, followed by the age group >19-29 with 16 cases, and the age group >29 with 10 cases. This finding is consistent with the results of the study conducted by Bhatti et al., (2019) which also showed a high representation of index cases in the age group of 9-19 years with congenital anomalies. Overall, understanding the distribution of cases based on socio-economic status and age groups is valuable for identifying potential risk factors for the occurrence of congenital and hereditary anomalies in different population subgroups.

In the study, the 201 index cases were analyzed based on parity, and it was observed that most index cases had first parity, accounting for 40% of cases. The second parity had the next highest representation at 22%, followed by the third parity at 13%. This finding aligns with the results of the study conducted by Mashhadi Abdolahi et al. in 2014, where most cases were in the first parity (50%), followed by the second parity (33%). Another study conducted in Chitral regarding congenital limb defects also found that the majority of cases belonged to the first parity (43%) (Malik et al., 2014). Similarly, Mahela (2016) reported the highest prevalence of anomalies in the first parity (31%), followed by the second parity (18%).

The study also examined the total familial subjects in terms of generation with the disease. It was found that most of the cases in the present study were segregating in one generation, accounting for 54% (n=43) of cases, while anomalies segregating in two generations constituted 46% (n=37) of cases. This result is consistent with the findings of

Zahra et al., (2016) who also reported the maximum number of diseases segregating in one generation.

This study may contribute to pattern and the self-reported risk factors of congenital anomalies in Mandi Bhauddin and may underscore the gaps in current knowledge about the causes of CA. Only the live infants were included in the study and stillbirths were excluded. The study was conducted in a district so does not truly represent the pattern and percentage of anomalies throughout the country. Furthermore, we could have underrated the risk factors and proportion caused by teratogenic exposure. Extensive studies to identify and determine the reasons for these defects and nation-wide screening studies are inevitable that investigate the birth prevalence, risks, types, and dissemination of birth defects.

Limitations

Indeed, like any research, this study has its own limitations that need to be acknowledged. First, the selection of the study population and the sampling method used may introduce biases and limit the generalizability of the results to the broader community. The data collection process could have been affected by certain factors, such as the reluctance of families of affected females to share their details, which may have impacted the study's completeness and accuracy. Furthermore, as this is a descriptive study, it can provide important associations between genetic factors and anomalies, but it cannot establish causality. To determine causality, more rigorous study designs, such as experimental or longitudinal studies, would be required. Additionally, the study's sample size may be limited, especially for rare anomalies, which could affect the statistical power to detect significant associations or relationships. A larger sample size would increase the study's ability to draw robust conclusions. Moreover, the study only includes cases of CA

that can easily be identified based on physical features, which might underestimate the true prevalence rate of congenital anomalies, as some milder or less apparent cases might be missed. Because data were usually taken from family or mothers or medical records of infants, there is a possibility that critical information for appropriate classification of defects was missing or unavailable at the time of enrollment.

Conclusion

This study highlights the dual role of genetics and environment in hereditary and congenital anomalies in Mandi Bahauddin's population. Consanguinity and sporadic cases underscore genetic and environmental influences, respectively. High prevalence of sporadic cases suggests that environmental factors have an important etiologic role in the study area and there is maximum potential for primary prevention. While this study has provided an initial overview, its scope was confined to a single district and a limited time frame. To elucidate a more specific picture of the patterns of congenital anomalies in this region, further extensive studies, with larger time frames and bigger study populations should be conducted.

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Performa ID:……..

Dated……

Parental details

Father/Mother Name	
Marital Ages Mother/Father	
Consanguinity	
Language	
Caste/Religion	
Economic Status/ Family type	
Parental education	
Contact Number	

Patient details

Name	
Age/Gender	
Marital Status	
Education	
Diagnosis	
Disease Onset	
No. of siblings Brothers/sisters	
Parity number	
Family history of anomaly	

Pregnancy details

Maternal age at subject birth	
Mode of delivery	
Complication during pregnancy	
History of miscarriage	
Medication during pregnancy	
Any treatment	
Baby cry at birth	

Phenotype of patient

Major presentation	
Weight	
Height	
Intellect	
Hearing	
Speech	
Vision	
Head Circumf.	
Limb defects R/L Arm/Hand Leg/Feet	
Any other details	

Medical record and Pictures taken?

Pedigree?